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# PHYTOPLANKTON IN VIRGINIA LAKES AND RESERVOIRS: PART II

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#### ABSTRACT

Phytoplankton composition from 16 Virginia lakes and reservoirs are discussed with emphasis on the dominant taxa, algal bloom producers, and potentially harmful species at these locations. This is a companion study to the more comprehensive publication regarding Virginia freshwater phytoplankton by Marshall (2013).

Keywords: phytoplankton, cyanobacteria, lakes, reservoirs, Virginia

#### INTRODUCTION

The phytoplankton composition from 16 Virginia freshwater habitats was examined as the continuation of a previous survey of 46 Virginia lakes and reservoirs by Marshall (2013). He identified 307 taxa considered representative species at these sites and which are also common in other U.S. north temperate lakes. The major bloom producers at these sites were cyanobacteria that included 6 potential toxin producing species. These were Anabaena circinalis, Anabaena spiroides, Aphanizomenon flosaquae, Cylindrospermopsis raciborskii, Limnothrix redekei, and Microcystis aeruginosa. There was concern that due to the aging nature of many of these and other freshwater habitats in Virginia, the likelihood of increased algal blooms occurring, including those by potentially harmful taxa, will become more common and extensive. The objectives of this study include the expanded coverage of Virginia lakes and reservoirs regarding their representative phytoplankton composition, their trophic habitat status, and to identify potentially harmful algal taxa among these populations.

#### **METHODS**

Between September 12-29, 2013, surface (<1m) water samples (1000 ml) were taken at 16 Virginia lakes and reservoirs (Fig. 1) not previously included by Marshall (2013). These sites were impoundments generally created decades ago. All water samples were preserved with Lugol's solution (1-2 ml) and returned to the Old Dominion University Phytoplankton Analysis Laboratory for examination. The Utermöhl method with an inverted plankton microscope was used to examine concentrated aliquots of these samples at magnifications of 300X and 600X (Marshall 2013). Cyanobacteria filaments were counted as numbers per ml, with colonial and

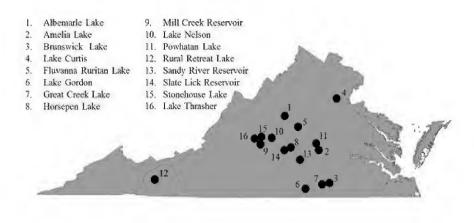


FIGURE 1. Locations of Virginia lakes and reservoirs where water samples for phytoplankton analysis were taken September 12-29, 2013.

unicellular cyanobacteria, and other algal species counted as number of cells per ml. Standard temperature and pH readings of the surface water were taken during each sampling.

#### RESULTS AND DISCUSSION

The 16 lake locations were centered predominantly in the Piedmont geographic region of Virginia. The Virginia Department of Game and Inland Fisheries indicated 8 of these lakes as eutrophic (Albemarle, Amelia, Curtis, Fluvanna Ruritan, Gordon, Horsepen, Stonehouse, Thrasher), 4 mesotrophic (Brunswick, Mill Creek, Nelson, Rural Retreat), and 1 oligotrophic (Slate), with 3 not designated (Brunswick, Powhatan, Sandy River). Neither of these non-designated lakes possessed high cell counts, and based on their algal composition appeared to be mesotrophic, but further nutrient analysis would be necessary to indicate their trophic status. Fifteen sites varied in size from 4 ha (Slate Creek Reservoir) to 85 ha (Great Creek Lake), with Sandy River Reservoir the largest in area at 299 ha. Surface water temperatures during sampling ranged from 25.5 to 30.5°C (mean 27.1°C), and pH ranged from 5.9 to 8.8 (mean 7.6).

The most common populations in these waters were cyanobacteria, chlorophytes, and diatoms. A variety of other taxa were also present, but generally in lower abundance and diversity. They included representative species of euglenophytes, cryptophytes, dinoflagellates, chrysophytes, and raphidophytes. These populations were similar to taxa previously reported by Marshall (2013), plus there were 35 additional taxa not identified in his previous collections (Table 1). In samples from these 16 sites, the chlorophytes included a diverse assemblage represented by several *Scenedesmus* and *Desmodesmus* taxa, plus *Staurastrum* spp., *Crucigenia* spp., *Tetraedron* spp., and *Chlorella* spp. The diatoms were common at each location, but generally not in high abundance during these collections. The exception was *Aulacoseira granulata* v. *angustissima*, with concentrations of 9,720 cells/ml in Amelia Lake. This taxon was

TABLE 1. Additional taxa to be added to phytoplankton recorded by Marshall (2013) in Virginia lakes and reservoirs.

#### Cyanobacteria

Aphanocapsa nubilum Komárek & Kling

Chroococcus distans (G.M. Smith) Komárkova-Legnerova & Cronberg

Chroococcus prescotti Drouet & Daily

Gomphosphaeria natans Komárck & Hindák

Oscillatoria lacustris (Klebahan) Geitler

Oscillatoria limnetica Lemmermann

Oscillatoria nigra Vaucher

Oscillatoria tenuis C. Agardh ex Gomont

Phormidium splendidum (Gomont) Anagnostidis & Komárek

Synechococcus nidulans (Pringsheim) Komárek & Bourrelly

Synechocystis aquatilis Sauvageau

Woronichinia fusca (Skuja) Komárek

#### Diatoms

Aulacoseira varians Agardh

Navicula capitata Ehrenberg

Nitzschia palea (Kützing) W. Smith

Synedra acicularis (Kützing) W. Smith

Synedra tenera Agardh

#### Chlorophytes

Chlorococcum minutum Starr

Desmodesmus hystrix Lagerheim

Scenedesmus acutus Meyen

Schizochlamys gelatinosa A. Braun

Sphaerocystis sp.

Stephanosphaera pluvialis Cohn

Stigeoclonium lubricum (Dillwyn) Kützing

Ulothrix tenuissima Kützing

Ulothrix zonata Kützing

#### Chlorophyte, Desmidiaceae

Closterium costatum Corda

Closterium sp.

Cosmarium tenue Archer

Cosmarium trilobulatum Reinsch

Xanthidium antilopaeum (Brébisson) Kützing

#### Chrysophytes

Dinobryon cylindricum Imhoff & Ettl

Dinobryon eurystoma (Lemmermann) Hillard & Asmund

#### Cryptophyte

Rhodomonas sp.

#### Dinoflagellate

Katodinium vorticella (Stein) Loeblich II

present throughout the sampling at each site. Other diatoms frequently noted included Fragilaria crotenensis and a variety of pennate taxa, plus several cryptophytes (e.g. Cryptomonas spp.) and a variety of euglenoids in low abundance, and all previously noted by Marshall (2013). In contrast, the cyanobacteria were generally more abundant than the other categories including having major bloom development in three of the eutrophic lakes (Table 2). These sites and the cyanobacteria bloom producers occurring there were Fluvanna Ruritan Lake (Jaaginema metaphyticum, Pseudanabaena acicularis), Lake Gordon (Cylindrospermopsis philippinensis, Glaucospira laxissima, Planktolyngbya contorta, P. limnetica), and Lake Thrasher (Microcystis aeruginosa, Anabaena viguieri).

Among the cyanobacteria were 4 potential toxin producing species. These taxa, along with their abundance and locations were Anabaena spiroides (Lake Amelia; 237 filaments/ml), Cyclindrospermopsis raciborskii (lakes Curtis, Fluvanna Ruritan, Thrasher, Sandy River; 60 to 11,520 filaments/ml), Limnothrix redekei (Lake Nelson; 1,020 filaments/ml), and Microcystis aeruginosa (Lake Thrasher; 25,820 cells/ml). Besides M. aeruginosa, other abundant cyanobacteria in Thrasher Lake, were Anabaena viguieri (8,640 filaments/ml), Planktolyngbya contorta (289 filaments/ml), and Planktolyngbya limnetica (290 filaments /ml). The other algal categories were less diverse and typically in low abundance. When present, these algal blooms produced a greenish coloration to the surface water with its intensity increasing with the bloom species abundance. However, during these collections, there were no indications of any harmful effects (e.g fish deaths) associated with any of the occurring bloom concentrations. Of note, numerous potentially harmful algal species have been reported in other water habitats in Virginia. For instance, several of these dominant and bloom producing cyanobacteria were in the low saline (oligonaline) waters of Back Bay Virginia (Marshall (2012), including bloom concentrations of Microcystis aeruginosa in the Potomac River (Marshall and Egerton 2009a). Other potential toxin producing dinoflagellates have been noted in Virginia tidal rivers and the Chesapeake Bay ecosystem (Marshall and Egerton 2009b, Marshall et al. 2009).

#### SUMMARY

A diverse assemblage of phytoplankton occurred in this sub-set of 16 Virginia lakes and reservoirs with a similar representation of dominant species and bloom producers to those previously reported in other Virginia freshwater locations by Marshall (2013). The sites included here are impoundments created over the past several decades possessing a range in size, with most at meso- to eutrophic stages of development. The water samples contained a broad representation of chlorophytes, diatoms, and cyanobacteria that included several bloom producing species and 4 potential toxin producers. With these 16 sites, this brings the total of freshwater habitats examined in this phytoplankton survey from 46 to 62 lakes and reservoirs, with a current phytoplankton listing raised to 342 taxa. Since these samples represented few sampling locations at each site and were not taken year-round, the collections are limited in scope, so further sampling will certainly expand the freshwater species list for these and other Virginia lakes and reservoirs; including species of potential toxin producing capability.

There continues to be concern regarding future transitions in the composition of these phytoplankton populations. These changes would accompany any increased Table 2. Abundance of bloom producing species in lakes Amelia, Gordon, Fluvanna Ruritan, and Thrasher recorded in September 2013.

#### Amelia Lake

Aulacoseira granulata v. angustissima 8,720 cells/ml

#### Lake Fluvanna Ruritan

Pseudanabaena acicularis 15,000 filaments/ml Jaaginema metaphyticum 11,400 filaments/ml

#### Lake Gordon

Cylindrospermopsis philippinensis 7620 filaments/ml Glaucospira laxissima 2,640 filaments/ml Planktolyngbya contorta 1,620 filaments/ml Planktolyngbya limnetica 6,720 filaments/ml

#### Lake Thrasher

Microcystis aeruginosa 25,820 cells/ml Anabaena viguieri 8,640 filaments/ml Planktolyngbya contorta 289 filaments/ml Planktolyngbya limnetica 290 filaments/ml

cutrophication at these sites associated with the natural aging of these habitats. This has direct relevancy to the older Virginia lakes and reservoirs where many of the less desirable species (e.g. cyanobacteria) would become more dominant, in contrast to algae generally considered the more desirable flora (e.g. chlorophytes, diatoms) as an oxygen and food source to various fauna in these waters. Projected climate change events would also influence the phytoplankton composition in these waters in a similar fashion (e.g. increased temperatures, rainfall, and nutrient entry, etc.) being more conducive to greater cyanobacteria development over other algae (Carey et al. 2012, Paerl and Paul 2012, Lürling et al. 2013). Thus, in future years, more Virginia lakes and reservoirs are predicted to have an increased presence and dominance of bloom producing taxa (specifically cyanobacteria), which may include many capable of toxin production. These results further support earlier findings by Marshall (2013) of the summer presence of several bloom and toxin producing species in Virginia's lakes and reservoirs.

#### **ACKNOWLEDGMENTS**

Appreciation is given to the Virginia Department of Health (VDH) for funding support regarding this study and Matthew Skiljo of VDH who collected the water samples examined in this study.

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#### Agriculture, Forestry, and Aquaculture

PREFERENCE OF HONEY BEES, CARPENTER BEES, AND BUMBLE BEES FOR NEW CROPS IN VIRGINIA. Mark Kraemer & Harbans Bhardwaj, Agricultural Research Station, Virginia State Univ., Petersburg, VA 23806. Agricultural crops may provide important nectar and pollen resources for native bees and the European honey bee, especially during the mid to late summer period when forage resources are limited. In some cases pollination is essential for good yields. Bee visits to several new crops were recorded during late August 2012 to replicated quarter acre plots of sesame, pigeon pea, mung bean, and lablab bean. Only 3 species constituted 99% of the bees visiting the flowers: carpenter bec (Xylocoris flavipes), common eastern bumble bee (Bombus impatiens), and European honey bee (Apis mellifera). Bees were counted during a 3-minute walk through of the field plots, repeated 3 times in a 3-week period. Significant differences (P<0.05) were found in the mean number of bees visiting the different crops using the PROC GLM procedure of SAS. Sesame attracted significantly larger numbers of B. impatiens (113) than either carpenter bees (1.8) or honey bees (0.1). In contrast, pigeon pea and lablab bean attracted significantly more carpenter bees (15.5 and 16.8, respectively) than bumble bees (2.8 and 1.0, respectively). Mung beans attracted significantly more bumble bees (12.0) and carpenter bees (4.7) than honey bees. Honey bee populations were low despite the presence of 10 active hives within ½ mile. The large number of B. impatiens in the sesame plots was likely partially due to their good physical fit within the corolla of the flower whereas carpenter bees were too large to enter the flower and those present attempted to rob nectar by chewing holes in the base of the corolla.

COMPARISON OF YIELD AND FRUIT SIZE OF FOUR RASPBERRY VARIETIES GROWN UNDER HIGH-TUNNEL CONDITIONS AT VSU'S RANDOLPH FARM. Jaylen Lewis, Reza Rafie, & Christopher Catanzaro, Dept. of Agriculture, Virginia State University. In the 2013 growing season a research project was conducted with four different raspberry varieties grown under high tunnel conditions. The objective of the project was to determine which variety produced the highest marketable yield and the largest fruit size. This study also measured the time period when the harvest initiated until it ended for each variety. The raspberry varieties planted for this research project were Himbo Top, Josephine, Joan J, and Polka. The raspberry fruits for each variety were harvested when the fruits ripened. The harvested fruits for each variety were weighted in grams and data was recorded. Fruit harvest initiated in July 2013 and continued until November of the same year. The raspberry variety Himbo Top produced 4.7 kilograms per plant in comparison to 3.7 kilograms per plant for Joan J, 3.7 kilograms per plant for Josephine, and 3.1 kilograms per plant for Polka. Variety Josephine on the average produced the largest fruit size, 48.4 grams for 10 fruits

weighted in comparison to 40.3 grams for HimboTop, 43 grams for Joan J, and 42.7 for Polka. The results from this research project showed that the raspberry variety Himbo Top produced the highest marketable yield and the variety Josephine produced the largest fruit size.

ORGANIC BLUEBERRY SWEETNESS: PERCENTAGE BRIX AND TASTE TESTS. Nicole R. Groff, Janaya M. Sachs, Tara L. Kishbaugh & Roman J. Miller, <sup>1</sup>Dept. of Biology and <sup>2</sup>Dept. of Chemistry Eastern Mennonite University. This study examined the effects of five different soil treatments and four different blueberry cultivars on the sweetness of blueberries. The soil treatments included Organic Horse Manure (OHM), Organic Sheep Manure (OSM), Organic Pinc Straw (OPS), Organic Planters Choice (OPC), and Conventional Horse Manure (CHM). The four cultivars included Bluccrop, Jersey, Bluegold, and Chandler. Sweetness was tested using %BRIX and taste tests, One %BRIX is approximately equal to 1g sucrose/ 100g solution. The berries grown in OHM had the sweetest berries, at an average of 13.3 %BRIX. Across cultivars, Jersey was the sweetest berry at average of 15.2 %BRIX. Two kinds of taste tests were administered: triangle and hedonic. The triangle taste test concluded that all the test subjects could tell a difference between OSM Chandler and OSM Jersey blueberry juice. The hedonic taste test concluded that Bluegold CHM was the least favorite and most tart sample across Bluegold OHM, OSM and CHM. Supported by USDA Specialty Crop Grant #2011-546.

BLUEBERRY ANTIOXIDANT ANALYSIS: SIGNIFICANCE OF SOIL TREATMENT AND CULTIVAR ON ANTIOXIDANT CONCENTRATIONS IN VACCINIUM CORYMBOSUM. M. Rhodes<sup>1</sup>, I. Pidroutchniak<sup>2</sup>, S. Cessna<sup>2</sup> & R. J. Miller<sup>1</sup>, <sup>1</sup>Dept. of Biology and <sup>2</sup>Dept. of Chemistry, Eastern Mennonite Univ., Harrisonburg VA 22801. Antioxidant concentrations are an important indicator of fruit quality. This project utilized the ferric reducing antioxidant power (FRAP) assay to determine whether variables such as fruit cultivar and soil treatment have a significant effect on antioxidant concentrations in samples of Bluecrop, Bluegold, Chandler, Duke, and Jersey cultivars of Northern Highbush Blueberries, Cultivars were grown in five different soil plots conditioned by incorporating specific mulches: OHM, organic horse manure; OSM, organic sheep manure; OPS, organic pine straw; OPC, organic planter's choice compost; or CHM, conventional horse manure. The FRAP assay revealed significantly higher antioxidant concentrations (micromoles/100g\*m²) in Bluegold and Duke cultivars grown in OPC than in Bluecrop, Chandler, and Jersey cultivars grown in the same plot (Bluecrop=1,966.74; Bluegold=3,208.24; Chandler=2,120.65; Duke=2,997.49; and Jersey=2,363.53). Significantly higher antioxidant concentrations were also observed in Bluccrop berries grown in plots mulched with OHM and OSM compared to plots mulched with OPS, OPC, or CHM (OHM=2,196.23; OSM=2,438.67; OPS=1,830.69; OPC=1,918.83; and CHM=1,871.82). This study was supported by USDA Specialty Crop Grant #2011-546.

VARIATIONS IN HIGHBUSH BLUEBERRY FRUIT SIZES AND QUANTITIES: INFLUENCES OF CULTIVAR SELECTION, PICKING TIMES, AND SOIL CONDITIONS. Roman J. Miller, Dept. of Biol., Eastern Mennonite University, Harrisonburg, VA 22802. Five blueberry cultivars (Duke, Bluecrop, Bluegold, Jersey,

and Chandler) were grown in five different soil plots conditioned by incorporating specific mulches: OHM, organic horse manure and sawdust; OSM, organic sheep manure and hay; OPS, organic pine needles and shredded bark; OPC, organic Planters Choice based on cow manure and fodder, and CHM, conventional horse manure and sawdust. In comparing 2013 versus 2012 harvests, productivity in the 2013 season was 87% greater (13.3 vs 7.1 cups/bush) and the average berry size was about 15% larger (95 vs. 110 berries/cup). Blueberry bush productivity in the 2013 season varied from high to low across the soil plots in order from OPS, OPC, OSM, CHM, and OHM (23.3, 17.1, 11.5, 8.5, and 7.5 cups/bush respectively). OPS and OPC productivity values were significantly higher than the rest. In comparing blueberry sizes among the different cultivars Chandler and Duke were significantly larger than the average (64 & 85 vs. 110 berries/cup). In comparing the production of the various cultivars Bluegold and Chandler tended to range above the average (18.4 & 15.7 vs. 13.3 cups/bush) while Jersey production was the lowest (10 cups/bush). In conclusion blueberry bushes in the OPS plot outperformed bushes in the other plots both in size and quantity. Supported by USDA Specialty Crop Grant #2011-546.

INVESTIGATING THE MOVEMENT AND HOST SELECTION OF MEXICAN BEAN BEETLE, EPILACHNA VARIVESTIS MULSANT, AMONG FIVE HOST PLANTS USING MARK-RELEASE-RECATURE. Louis B. Nottingham & Thomas P. Kuhar, Dept. of Entomology, Va. Polytechnic Inst. & State Univ., Blacksburg VA 24061. Mexican bean beetle, Epilachna varivestis Mulsant, feeds upon various bean crops in the United States. The level of suitability among many of these crops is described in previous research; however, little is known about how MBB exhibits preference when numerous host plants are available in one setting, such as a polyculture farm. Our research examined the movement and host selection of MBB among common host plants using mark-release-recapture field trials. Experiments were conducted in a field plot, as well as inside a walk-in screen cage, located near Blacksburg, VA in 2013. In both experiments, 250 adult MBB were released into a plot planted with five replicates of five bean varieties (purple wax snap, yellow wax snap, green snap, lima and soy). Each beetle was painted with a color indicating the host plant on which it was released. Plots were sampled every two days until recapture rate dropped to 10%. Our data revealed that MBB movement was not random, even among the most suitable hosts (snap beans). More MBB originally released in purple wax beans remained in this crop. Also, more MBB move into purple wax beans than any other crop. This information will aid in the development of cultural management strategies for this pest.

INSULIN-INDUCED HYPOGLYCEMIA IS ASSOCIATED WITH GENE EXPRESSION CHANGES IN THE LIVER AND HYPOTHALAMUS OF CHICKENS SELECTED FOR LOW OR HIGH BODY WEIGHT. B. B. Rice, W. Zhang, S. Bai, P. B. Siegel, M. A. Cline & E. R. Gilbert, Dept. of Animal and Poultry Sci., Va. Polytechnic Inst. & State Univ., Blacksburg VA 24061. Chickens selected for low (LWS) or high (HWS) body weight for more than 56 generations now show a 10-fold difference in body weight and correlated responses in appetite and glucose regulation. The LWS chickens are lean and some are anorexic, while the HWS are compulsive feeders and display lower threshold sensitivity to both central and

peripheral insulin. We previously demonstrated that at 90-days of age insulin-induced hypoglycemia was associated with reduced glucose transporter expression in the liver of both LWS and HWS chicks, and differences in appetite regulatory genes between LWS and HWS in the hypothalamus. The objective of this study was to determine effects of insulin-induced hypoglycemia on gene expression in the hypothalamus and liver of early post-hatch LWS and HWS chicks, before the onset of obesity in HWS chicks. Five-day old LWS and HWS chicks were fasted for 3 hours and injected intraperitoneally with insulin or vehicle. At 1 hour post-injection, chicks were euthanized, blood glucose measured, and hypothalamus and liver removed. Total RNA was isolated and real time PCR performed. Results are consistent with studies conducted in older chickens.

ELUCIDATING THE OREXIGENIC MECHANISM OF PROLACTIN-RELEASING PEPTIDE IN CHICKS. Guoqing Wang, Elizabeth R. Gilbert & Mark A. Cline, Department of Animal and Poultry Science, Virginia Polytechnic and State University, Blacksburg, VA 24061. Prolactin-releasing peptide (PrRP) is an endogenous hypothalamic peptide that when exogenously administered causes decreased food intake in rats; however, when administered to chicks it causes a potent increase in food intake. The central mechanisms mediating the appetite-associated effects of PrRP are poorly understood in any species, especially so in chicks. Thus, our purpose was to further elucidate the orexigenic mechanism of PrRP in chicks. Chicks that received intracerebroventricular (ICV) injection of PrRP at a dose as low as 3 pmol increased food intake up to 60 min following injection. Those treated with higher doses, up to 188 pmol, increased food intake throughout the entire 180 min observation period while water intake was not affected by any dose. Whole hypothalamus was collected from 188 pmol PrRP-injected chicks, total RNA isolated, reverse transcribed, and real-time PCR performed. Chicks that received ICV PrRP injection had decreased hypothalamic oxytocin and orexin mRNA abundance compared to the vehicle-injected chicks. In conclusion, ICV PrRP injection increases food intake in chicks and is associated with decreased expression of hypothalamic oxytocin and orexin mRNA.

ELUCIDATING GONADOTROPIN-INHIBITORY HORMONE'S MECHANISM OF HUNGER STIMULATION. Betty R. McConn<sup>1</sup>, E. R. Gilbert<sup>1</sup>, M. Furuse<sup>2</sup>, V. Chowdhury<sup>2</sup>, M. A. Cline<sup>1</sup>, G. Wang<sup>1</sup> & J. Yi<sup>1</sup>. <sup>1</sup>Neuroendocrinology Laboratory, Va. Polytechnic Inst. & State Univ., Blacksburg VA 24061 and <sup>2</sup>Laboratory of Advanced Animal & Marine Bioresources, Kyushu University, Japan. Gonadotropin-inhibitory hormone (GnIH) was first isolated from the quail brain and was found to be localized in the periventricular nucleus of the hypothalamus. We designed the study to further investigate the central mechanism of GnIH's orexigenic effect in chicks with a focus on the hypothalamus. We performed intracerebroventricular (ICV) injections of 0, 0.9, 2.6, 7.8 nmol GnIH to ad libitum fed 14 day post-hatch White Leghorn chicks and found that 2.6 and 7.8 nmol doses increased food intake. On a cumulative basis this effect persisted for 150 min following injection, while none of the doses affected water intake. Following this, we ICV administered 2.6 nmol GnIH and measured c-Fos immunoreactivity in key hypothalamic nuclei associated with appetite where only the lateral hypothalamus (LH) increased activation. Next, we took the whole hypothalamus and mRNA expression showed an increase in neuropeptide Y (NPY) and a decrease in

pro-opiomelanocortin (POMC). Lastly, in the LH isolated from GnIH-injected chicks, there was an increase expression of melanin-concentrating hormone (MCH). Thus, the orexigenic effects of GnIH appear to involve the LH and affect NPY, POMC, and MCH. Supported by Virginia Academy of Sciences Undergraduate Grant Award.

THE SCIENCE OF VIRGINIA'S AQUACULTURE FEED PRICES. Brian L. Nerrie, Virginia Cooperative Extension, Virginia State University, Petersburg, VA 23806. Aquaculture feed costs are between 50-60% of the operating expenses. Aquaculture's growth has resulted from improved knowledge of the nutritional requirements of aquacultured products. Initially nutrients were supplied by natural pond foods, and later by enhanced fertilization to improve pond productivity. Aquaculture feeds were developed that enabled farmers to provide uniform-size products to satisfy consumer demand for high quality affordable protein. Small changes in feed prices result in larger influences on product cost. Feed grains are a major component of aquaculture feeds. Increases in feed prices have resulted from alternative uses of acreage used for feed grain production especially biofuel development, decreased supply due to international demand for feed grains and local transportation costs. Economies of size in Virginia's aquaculture industry add to prices.

#### Astronomy, Mathematics, and Physics with Material Science

CALCULATION OF THE ELECTRON MASS FROM ITS FIELDS. J. D. Rudmin, The College of Integrated Science and Engineering, James Madison University, Harrisonburg, VA 22807. The masses of the three flavors of the electron are calculated from the electroweak fields. A normal charge distribution provides field reduction at short range. Lagrange's equations are solved exactly or by polynomial approximation (The Parker-Sochacki method). If a term is included which has the form of a magnetic moment, with the mass of the W for the mass of the magnetic moment, then the electron, muon, and tau have evenly spaced masses in natural units. The even spacing of mass suggests that the electron has one unit of flavor that couples to the Higgs field like a spin-1 magnetic moment couples to a magnetic field. The electron then adjusts its size for a self-consistent mass. Since the electron is a fermion, it would contribute 1/2 unit of flavor. Perhaps the local direction of time contributes the additional 1/2 unit of flavor.

TEMPERATURE-AND TIME-RESOLVED SPECTROSCOPY OF CuUnS<sub>2</sub> SEMICONDUCTOR NANOCRYSTALS WITH ZnS CAPPING. Quinton Rice<sup>1</sup>, Sangram Raut<sup>2</sup>, Ignacy Gryczynski<sup>2</sup>, Zygmunt Gryczynski<sup>2</sup>, Bagher Tabibi<sup>1</sup>, & Jaetae Seo<sup>1</sup>, <sup>1</sup>Advanced Center for Laser Science and Spectroscopy, Department of Physics, Hampton University, Hampton, Virginia 23668 and <sup>2</sup>Center for Commercialization of Fluorescence Technologies, Department of Cell Biology, University of North Texas Health Science Center, Fort Worth, Texas 76107. Semiconductor nanocrystals (SNCs) have been a prominent research area for photonic applications of optoelectronic devices, light emitting devices (LEDs), and biomedical applications due to their high quantum yield, wide tunability, and ability to be integrated with micro-photonic and electronic devices. Also, the SNCs of CuInS, do not possess intrinsic toxicity

associated with those of heavy metals such as cadmium or lead chalcogenide which limit practical biomedical applications. Generally, the SNCs with sizes near the bulk Bohr radius display quantum characteristics which are confirmed by the strong optical blue-shift from the band gap of bulk materials and the discrete energy states that develop from confined electron-hole pairs within the size limit of the nanocrystal. The SNCs are highly sensitive to morphology, composition, and lattice strains or defects in the structure. This work includes time-resolved and temperature-dependent optical properties that revealed several characteristics linked to surface-trapped state recombination and shallow or deep defect related donor-acceptor transitions which are widely distributed throughout the entire emission spectra. The temperature-resolved photoluminescence showed that the emission from surface-trapped and interface-trapped transitions remained thermally active at low temperatures while emissions from intrinsic defect related transitions were strongly quenched due to their stability from the Coulomb interaction. Acknowledgement: This work at HU is supported by NSF HRD-1137747 and ARO W911NF-11-1-0177.

TIME-RESOLVED COLORIMETRIC FLOCCULATION AND SURFACE-ENHANCED RAMAN SCATTERING OF MOLECULE-LINKEDPLASMONIC NANOPARTICLES. A. Hayes, Q. Rice, M. Abdel-Fattah, W. Kim, S. Jung, B. Tabibi, & J. Seo', Advanced Center for Laser Science and Spectroscopy, Department of Physics, Hampton University, Hampton, Virginia 23668, U.S.A, <sup>2</sup>Biosensor Research Team, Electronics and Telecommunications Research Institute, Daejeon 305-700, South Korea and Korea Research Institute of Standards and Science, Daejeon 305-600, South Korca, Surface-enhanced Raman spectroscopy (SERS) has been of great interest because of large enhancement of molecule Raman scattering under strong plasmonic field. The SERS provides the finger print of molecule information with signal enhancement. The colorimetric flocculation indicates the molecule linkages on the plasmonic nanoparticles. The spectral coupling between the localized surface plasmonic resonance (LSPR) at longer wavelength and laser frequency efficiently excites the Raman molecules. The molecule-linked plasmonic nanoparticles form a hot spot where the Raman analytes are located. The Au nanoparticles are prepared with average diameters of ~5, ~10, ~15, ~20, ~30, ~32, ~35, and ~40 nm within the plasmonic dipole approximation at the optical spectral region. The time-SERS is for 0, 2, 5, 10, 20, 30, 60, 120 and 180 minutes after the molecule was mixed with plasmonic particles. The colorimetric flocculation of molecule-linked plasmonic particles was monitored at same time. This presentation includes colorimetric flocculation and SERS of molecule-linked plasmonic particles with synthetic urine for biomedical applications. Acknowledgement: The work at HU was supported by NSF HRD-1137747 and ARO W911NF-11-1-0177.

MULTI-WAVELENGTH ANALYSIS OF THE EMPIRICAL SERKOWSKI RELATIONSHIP FOR INTERSTELLAR POLARIZATION. G. E. Matthews & G.A. Topasna, Department of Physics and Astronomy, Virginia Military Institute, Lexington, VA 24450. Using broadband filters, the wavelength dependence of polarization in the visual is described by the Serkowski relationship  $p(\lambda)/p_{\text{max}} = exp[-K \ln^2(\lambda_{\text{max}}/\lambda)]$  where  $K \approx 1.7 \lambda_{\text{max}}$  and is empirically well-established for stellar observations. We confirmed the validity of the Serkowski relationship for stellar observations using narrowband

filters by making polarimetric observations of the star HD 197770 using both narrowband ( $\Delta\lambda\sim10$  nm) and Johnson-Cousins UBVRI broadband ( $\Delta\lambda\sim100-200$  nm) filters. The wavelength dependence of polarization for the two different filter sets was found and a nonlinear least square fit to the Serkowski relationship determined the maximum degree of polarization,  $p_{\rm max}$ , and the wavelength of maximum polarization,  $\lambda_{\rm max}$ . We found that the broadband filters had a maximum polarization of  $4.14\pm0.02$ % at a wavelength of  $527\pm2$  nm and the narrowband filter's maximum polarization was  $4.033\pm0.023$ % at  $532\pm6$  nm. The percent difference between broad and narrow band filters was 0.88% and the difference between their maximum polarizations was on the order of the instrumental polarization at 0.1%. Our findings show narrowband filters exhibit an identical Serkowski relationship to broadband filters. This is significant because it allows astronomers to now utilize the smaller band-pass of narrowband filters to image stars in regions of high nebulosity, producing more accurate and precise images than broadband filters are capable of.

EXTINCTION IN DISCRETE COMPETITIVE MULTI-SPECIES PATCH MODELS. D. M. Chan, M. D. McCombs, S. Boegner, H. J. Ban & S. Robertson, Department of Mathematics & Applied Mathematics, Virginia Commonwealth University, Richmond, VA 23284. In this paper, we extend the results of Franke and Yakubu for extinction in discrete competitive patch models. For a system of n species on m patches, we define conditions under which one species is a "superior competitor" to another and show that it is sufficient for one species to drive another to extinction. We also illustrate the result with an example for three species on three patches.

INFRARED AND THERMOELECTRIC POWER GENERATION IN THIN ALD THERMOELECTRIC FILMS. Harkirat S. Mann', Brian N. Lang', Yosyp Schwab', Janne Niemelä<sup>2</sup>, Maarit Karppinen & Giovanna Scarel<sup>1</sup>, <sup>1</sup>James Madison University, Department of Physics and Astronomy, Harrisonburg, VA 22807, USA <sup>2</sup>Aalto University, Department of Chemistry, FI-00076 Aalto, Finland. A mechanism for alternative energy, thermoelectric (TEC) power generation, converts a temperature difference across two junctions into an electric potential. Although not as energyefficient as solar panels or wind turbines, this mechanism is used in a wide variety of fields, e.g. to recapture waste heat. Recently it was discovered that a solid state TEC power generators respond differently to heat or infrared (IR) radiation. To test the robustness of this finding, this research compares TEC and IR power generation in the case of a nanometric TEC device in which the active element is a thin TEC film. The thin TEC film is a 70 nm thick n-type Nb-doped titanium oxide film deposited by atomic layer deposition (ALD) onto a borosilicate glass substrate. The interactions observed with heat show a linear relationship between temperature and voltage, whereas in IR radiation this linear relationship is broken down.

#### Posters

FABRICATION, CHARACTERIZATION, AND CONTROLLED ASSEMBLY OF POLYSTYRENE BASED JANUS PARTICLES. <u>D. M. Topasna</u> & G. A. Topasna, Department of Physics and Astronomy, Virginia Military Institute, Lexington, VA 24450. We present results on fabrication and controlled assembly of Janus particles.

The Janus particles were fabricated from  $21 \mu m$  polystyrene spheres deposited on glass slides and covered with a top aluminum thin film. These Janus particles were then deposited in a specially fabricated cell and their alignment was controlled with AC electric fields at low frequency of 50 Hz.

POLARIMETRIC OBSERVATIONS OF HD 197770. G. A. Topasna, Department of Physics and Astronomy, Virginia Military Institute, Lexington, VA 24450. Observations of the polarized star HD 197770 were made in the V band using the using the optical polarimeter on the 0.5 meter telescope at the Virginia Military Institute observatory located at McKethan Park in Lexington, VA. Observations of other standard stars were made for comparison of the degree of polarization and the polarization position angle. The analysis shows that the HD 197770 shows less variability than the other standards stars. The mean values of the normalized Stokes parameters q and u were analyzed and the null hypothesis of equality of population means was tested using the Welch test. The weighted average of the normalized Stokes parameters were used to calculate the degree polarization and position angle and found to be  $4.087 \pm 0.003$  % and  $131.16 \pm 0.05$ ° respectively. Given the stability of HD 197770 we recommend that it be studied further across multiple wavelengths for possible use as a secondary standard star.

OPEN SOURCE TEXTBOOKS. Thomas C. Mosca III, Rappahannock Community College, Department of Mathematics, Warsaw, VA 22572. With textbooks costing hundreds of dollars per class, there is considerable motivation to examine "open educational resource" (OER) materials. This presentation provides a brief introduction to open source teaching materials for math and physics classes, and touches briefly on copyright. The purpose of this talk is more to stimulate discussion of the pros and cons, and also to solicit sources from members of the audience, for the benefit of other members.

#### Biology with Microbiology and Molecular Biology

INTIMATE ATTACHMENT: THE INTIMIN STORY OF PATHOGENESIS. Abigail Lenz, Department of Biology and Chemistry, Liberty University, Lynchburg VA 24502. Enterohemorrhagic Escherichia coli (EHEC) is a bacterium causing of mortality via hemolytic uremic syndrome. The virulence EHEC causes in humans is modeled in mice with Citrobacter rodentium (CR), which is a normal murine pathogen causing transmissible murine colonic hyperplasia but is not pathogenic to humans. The two most widely used strains of CR are ICC168 and DBS100, but only ICC168 has been sequenced. Therefore, genetic differences between these widely used strains have yet to be determined. We sequenced DBS100 and found it to be different from its presumably clonal ancestor by almost 400,000 nucleotides. Colonization strategies are thought to be among genetic difference in strains of CR. The process of attachment is important to colonization and involves adhesins, the primary of which is intimin in EHEC and CR. An intimin mutation divorces attachment from pathogenesis in colonization, thus addressing the importance of competition in pathogenesis. Similarly, the introduction and removal of antibiotics in the course of colonization creates a

competitive environment. We found that a more competitive environment increased pathogenesis of CR in the murine intestine.

INTERACTIONS BETWEEN NANOPARTICLES AND MACROPHAGES. M. Bani-Hani', C. Osgood' & M. Stacey<sup>2</sup>, 'Department of biological sciences, Old Dominion University, Norfolk VA 23529 and Frank Reidy research center for bioelectrics, ODU, Norfolk VA 23529. The unique characteristics of Nanoparticles (NP) make them suitable for a wide range of applications. However, the use of NP raises concerns regarding their potential impact on health. NP could lead to enhanced or suppressed immune functions and they may damage unintended target tissues. To assess the safety and potential impact of NP, we investigated interactions between macrophages and nanocapsules (NC), We used mouse cell line, J774A,1, incubated with different concentrations of NC. The NC were tagged with near infrared (NIR) NC suitable for live animals imaging and flow cytometry analysis. The same NC were tagged with Alexa-488 Fluor for microscopic analysis. The flow cytometry analysis showed the increased fluorescence from cells incubated with these NC for up to 5 hours. From the microscopy images, we found that most (but not all) macrophages engulfed the NC in a time- and dose-dependent manner and most of these NC were localized to the cytoplasm. In addition, some NC entered the nucleus which was clear from the DAPI stain after the NC treatment. Cell viability were not affected by the NC even with the higher NC concentrations and for longer times as it appeared from the MTT assay. Our results, in addition to some preliminary data about NC toxicity, indicated that these NC are not killing the cells but may induce macrophage response. Our next work investigates macrophage function in response to NC treatment.

INDUCTION OF P53 ACTIVITY IN NIH 3T3 CELLS FOLLOWING EXPOSURE TO NON-IONIZING RADIATION. Yoshinori Takeda & Rosemary Barra, Dept. of Biology, University of Mary Washington, Fredericksburg, VA 22401. Radiation biology has been of interest in recent years with the primary focus on ionizing radiation and its effects on DNA. However, there is another, larger part of the electromagnetic spectrum, the non-ionizing radiation section. Recent articles suggest that free radical oxygen forming reactions are possible with exposure to 60Hz frequencies, and that p53 expression is increased in cells exposed to infrared radiation. In this study, NIH 3T3 cells were exposed to a helium-neon laser (4mW, 632.8nm) for 3-5 hours for 2-5 days. To observe the effects by the laser, p53 expression and radical oxygen presence were measured as indicators of DNA damage or response to DNA damage. Two-way ANOVA was performed to test for significance on hours of exposure, radiation exposure versus control, and effects of distance on the treated cells grown in a slide chamber. p53 expression was found to be significantly higher (23% increase in expression of p53) in radiation-treated cells compared to the control (N=28, p=0.028). Radical oxygen levels were not significantly different (N=6, p>0.05), but this may be due to a small sample size and warrants further study. Overall, the data suggests that non-ionizing radiation affects fibroblast cells and p53 expression. Further studies will focus on various radiation sources and the mechanism for p53 activation following exposure to non-ionizing radiation.

IFNL4 GENE EXPRESSION IN HEPATITIS C VIRUS INFECTION. Kellie Perry, 1,2 Ancha Baranova<sup>1,2</sup> & Michael Estep<sup>1</sup>, <sup>1</sup>Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church, VA, <sup>2</sup>Center for the Study of Chronic Metabolic Diseases, George Mason University, Fairfax, VA. HCV is associated with long term chronic health conditions such as cirrhosis of the liver, cancer of the liver, and is the primary reason for liver transplants. Our aim was to examine the expression of interferon response genes during IFN+Ribavirin treatment in the context of the IFNL4 genotype. IFNL4 is a SNP that induces an immune response in the presence of HCV infection occurs via activating the Janus kinase signal transducer and activator of transcription (STAT) pathway. The rs368234815 gene that encodes for INFL4 and its variants are located on chromosome 19q13 just upstream of the (rs12979860) IL28B gene that encodes for IFNL3. There are two genetic variants of IFNL4, "?G" and the other nonsense mutation "TT". IFNL4 (rs368234815) has been linked with inducing an immune response and sustained virologic response (SVR) like its neighbor IFNL3 (IL28). In this study, IFNL4-TT showed a strong linkage to IL28B-C allele. Gene expression increased with IFNL4 associated genes on Day 0 and Day 7. Expression differences were observed with IFNL4 associated genes linked to IFNg pathway associated genes on Day 0 and Day 7. The top 5 pathways linked to IFNL4 were apoptotic pathways. Decreased expression with apoptotic genes were correlated with the nonsense IFNL4-TT allele. Relevant time points of gene expression differences were Pretreatment and Day 7. Pathways associated with IFNL4 were predominately apoptotic.

EPIGENETIC ALTERATIONS IN A TRANSGENIC ALZHEIMER'S DISEASE MODEL. Matthew Baker, Michael Carson, Rebecca Haraf, Noor Taher, Amanda Hazy & Gary D. Isaacs, Dept. of Biology and Chemistry, Liberty University, Lynchburg VA. Genetic contributions leading to the development of Alzheimer's disease are mostly obscure, however evidence suggests that epigenetic modifications may play a role. Cytosine methylation has been shown to play a role in several biological disease states, and several AD-associated genes are regulated through this mechanism. Therefore, we set out to determine if methylation can provide genetic markers for AD development. The methylation levels of DNA promoter regions from transgenic mice, which overexpress beta amyloid as seen in AD, were compared to that of control transgenic mice. Two methodologies were used for determining promoter methylation statuses. First, using methylation sensitive (HpaII) and insensitive (MspI) restriction endonucleases, which both target the sequence 5'-CCGG-3', we determined the methylation status of genomic promoter regions through LM-PCR and subsequent microarray analysis. These genomic regions showing Aβ-induced methylation modifications were then narrowed down to the 0.1% most changing regions for stringeney purposes. The other methodology used was Methylative DNA Immunoprecipitation, which uses antibodies to isolate methylated genomic fragments that are then hybridized to a microarray. The regions undergoing significant change were linked to their ontological function, revealing correlation with ontologies related to neurogenesis and apoptosis. This evidence seems to indicate an epigenetic contribution to AD pathology. This work is supported by the Jeffress Memorial Trust (Grant J-998), the ARDRAF Grant, and the Virginia Academy of Science.

DETERMINING THE RATIO OF THE MITOCHONDRIAL DNA IN BROWN ADIPOSE TISSUE TO WHITE ADIPOSE TISSUE, Amir Mohammadhasani<sup>2</sup>, Maria Keaton<sup>2</sup>, Lei Wang<sup>2</sup>, Zobair M. Younossi<sup>1,2,3</sup>, Ancha Baranova<sup>1,2</sup>, Aybike Birerdinc<sup>1,2</sup>. <sup>1</sup>Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church, VA, <sup>2</sup>Center for the Study of Chronic Metabolic Diseases, George Mason University, Fairfax, VA, 3Center for Liver Diseases, Department of Medicine, Inova Fairfax Hospital, Falls Church, VA. Many recent studies show obesity is now a global public health problem due to the increase in mortality associated with increasing BMI. In adults, visceral adipose is mostly comprised of lipid storing white adipose tissue (WAT) with limited amounts of lipid expending capable brown adipose tissue (BAT) which may play a role in obesity induced chronic diseases. Due to the high number of mitochondria expressing UCP1 BAT cells are capable of using lipids to produce heat. RNA and DNA was extracted from visceral adipose samples collected from obese patients during elective gastric bypass. Mitochondrial and genomic DNA were compared in ratios. Relative expression of PRDM16 and UCP1 were measured with validated primers. Spearman's correlation reveals UCP1 and PRDM16 are strongly positively correlated (r= 0.6032, p<4.035e-09). However the genomic and mitochondrial ratios were could only be analyzed within a range since BAT activation is regulated by low temperatures. Further research will include differentiation of hTERT immortalized adipose derived Mesenchymal stem cells into BAT and WAT to use as positive and negative ends of the ratio spectrum.

UTILIZING NONTHERMAL PLASMA BASED ON SHIELDED SLIDING DISCHARGE IN AMBIENT AIR FOR DELIVERY OF PLASMID DNA TO MAMMALIAN CELLS. C.M. Edelblute<sup>1,2</sup>, L.C. Heller<sup>1,3</sup>, M.A. Malik<sup>1</sup>, & R. Heller<sup>1,3</sup>, Frank Reidy Research Center for Bioelectrics, Old Dominion University, Norfolk, VA,<sup>2</sup> Department of Biology, Old Dominion University, Norfolk, VA, and <sup>3</sup> Medical Diagnostic and Translational Sciences, Old Dominion University, Norfolk, VA. In the current study, we assessed the efficacy of a novel non-thermal plasma reactor based on shielded sliding discharge (SSD) producing cathode-directed streamers generated in ambient air for the delivery of plasmid DNA to mammalian cells. In vitro experiments were performed with mouse melanoma cells (B16F10) and human keratinocytes (HaCaT) inoculated with plasmid DNA encoding luciferase (pLUC) and green fluorescent protein (pGFP). Quantitative results measured over a 72-hour period displayed luciferase expression levels as high as 10-fold greater in SSD plasmaexposed cells than levels obtained from the inoculation of plasmid DNA alone (p<0.05, p<0.01). No effect on viability was observed as consequence to plasma exposure in either cell line. SSD plasma-mediated pGFP delivery to HaCaT cells seeded on polycaprolactone scaffolds was confirmed by immunostaining. SSD plasma exposure significantly enhanced pLUC expression in an in vivo murine model over 28 days compared to intradermal pLUC injection alone (p<0.05). Our findings suggest SSD plasma is an attractive non-viral, non-contact DNA transfer method that warrants further exploration as an alternative or supplemental molecular delivery approach.

POSITIVE SELECTION OF SPERMATOGONIAL STEM CELLS. <u>Eoin C. Whelan¹</u>, A. Nwala², S. Arif⁴, S. Olariu² & C. Osgood¹, ¹Department of Biology ODU, Norfolk VA 23508, ²Department of Computer Science ODU, Nofolk VA 23508. The paternal

age effect is when the incidence of certain genetic diseases is associated with father's age. A model has been proposed that explains the exponential increase in disease incidence with father's age via the positive selection of mutations in spermatogonial stem cells while in the testes. We developed a computer model that simulates the stem cell division within the niche and accounts for mutation accumulation in a stochastic manner. The goal was to match the model to existing clinical data and estimate the minimum selection pressure required to explain the disease incidence rates. Our model assumed homeostasis of the stem cell niche and that each seminferous tubule represented a single niche. Each niche began with zero mutant cells. We estimated mutation rate based on the average human mutation rate and the rate of spermatogonial stem cell division. The selective advantage was the probability assigned that if a mutant cell divides it will undergo a symmetric division and produce an additional mutant stem cell. Each run of the simulation showed clumping of mutations consistent with the selective model. The data from the model output was averaged and used to produce a simulated population in order to compare the model to the clinical data. The model agreed with data from clinical studies of paternal age effect syndromes with a selective advantage of 0.005 to 0.01. The paternal age effect incidence rate could not be explained by the model with mutation rate alone but by adding a selective advantage to the mutations our data closely aligns to the clinical incidence. (Supported by Sigma Xi Grant-in-Aid of Research and Virginia Academy of Science)

UTILIZATION OF ENTERIC SIGNAL AUTO-INDUCER 3 BY ESCHERICHIA COLI TO REGULATE MOTILITY OF FLHDC THROUGH QUORUM SENSING. <sup>1</sup>Ryan N. Montalvo & <sup>2</sup>E. D. Richards, Liberty University <sup>1</sup>Department of Biology and Chemistry & <sup>2</sup>Department of Health Sciences. The interplay of enteric bacteria involves communication utilizing quorum sensing mechanisms. Escherichia coli regularly utilize the histidine kinase receptor QseC to regulate motility in the flhDC operon. OseC autophosphorylates in response to stress signals within the intestine and is stimulated by the adrenergic hormones epinephrine and norepinephrine as well as a quorum sensing molecule AI-3. The producer(s) of AI-3 within the intestine for E. coli are unclear, qPCR of fecal samples quantified concentrations of intestinal bacteria for streptomycin-treated mice and streptomycin-treated mice colonized with E. coli MG1655, HS, and Nissle, Bifidobacterium display fold increases of 7.5, 6.6, and 11 respectively, at 7 days post-infection. Our results implicate Bifidobacterium spp. in the changing motility phenotype. Over a 60-days, we observe population instability in relation to motility and the flhDC locus. Sequencing reveals analogous deletions within the flhDC loci of non-motile MG1655, HS, and Nissle that. Through motility assays, we observe that MG1655, HS, and Nissle fluctuate from 100% motility to 20-60% motility at 60 days. While the HS and Nissle strains lack the same promoter as MG1655, they retain the capability to exhibit non-motility phenotypes. Distinct mechanisms regulate flhDC. Murine and biosynthetic colonization with MG1655, HS, and Nissle strains display the nature of bacterial population fluctuations within the intestine that help define colonization drifts over time.

SEASONAL SHIFTING OF THE TROPHIC NICHE IN A GENERALIST APEX PREDATOR. P. A. P. deHart<sup>1</sup>, L.E. Hurd<sup>2</sup>, J.M. Taylor<sup>2</sup>, M.M. Shearer<sup>2</sup>, & M. C. Campbell<sup>2</sup>, <sup>1</sup>Department of Biology, Virginia Military Institute, Lexington VA 24450

and Biology Department, Washington & Lee University, Lexington VA 24450. Apex predators influence community composition and diversity through direct and indirect interactions with other species, but they may not occupy the top trophic level throughout their lives. The question remains: are top predators feeding in a frequencydependent manner, or are they varying their prey in accordance with different dietary demands as they grow and mature? We investigated this question by examining the feeding relationships throughout the lifespan of a well-studied apex predator, the praving mantis Tenodera aridifolia sinensis, using stable isotope analysis. We measured the δ<sup>13</sup>C and δ<sup>15</sup>N of lab-reared mantids fed known diets of arthropods from the key feeding guilds (leaf chewers, sap feeders, and carnivores), of their prey items, and of field-caught mantids over a growing season, Lab-raised mantids generally increased in both  $\delta^{13}$ C and  $\delta^{15}$ N with the consumption of successively higher trophic level prey, but field-caught mantids were highly variable in this regard. These signatures indicated that mantids did not take prey from trophic guilds in proportion to their abundances, so are not frequency-dependent predators. There was a clear pattern of 15N enrichment during growth and development of mantids, with adults and eggs exhibiting highest values, decreasing from egg hatch to third instar as nymphs fed on lower trophic levels and increasing steadily thereafter, indicating increased carnivores in the diet as nymphs grew. Our results suggest that variability in the community impact of apex generalist predators is strongly influenced by ontogenic shifts in dietary demand.

#### Posters

PHYLOGENETIC ANALYSES OF STREPTOCOCCUS PARAUBERIS FROM FISH AND CATTLE STRAINS. Keaira L. Thornton & Ashley N. Haines, Dept. of Biology, Norfolk State University, Norfolk, Va. 23504. Streptococcus parauberis is a gram positive lactic acid bacterium that is found locally and globally and recently has become an emerging pathogen. S. parauberis infects cattle and fish, which can negatively impact dairy and aquaculture fisheries. The genomes of multiple isolates from fish were sequenced and compared to the available published genomes from one fish and one cattle isolate. The genomic sequences of various housekeeping genes were used to construct phylogenetic trees using the software Geneious 6.1.5 via Neighbor-Joining and Unweighted Pair Group Method with Arithmetic Mean methods. These phylogenetic analyses will contribute to our understanding of the evolutionary relationships between the strains of this bacterium in different animal host species and predict the closest relative to S. parauberis. These data will help explain the molecular epidemiology of S. parauberis and help us understand how it spreads from host to host and why it is becoming an emerging pathogen.

PREPS ANALYSIS OF VIRAL PROTEINS POTENTIALLY PRENYLATED BY HUMAN CELLS. Ekateina Marakasova<sup>1</sup>, Birgit Eisenhaber<sup>2</sup>, Frank Eisenhaber<sup>2</sup>, Elena Yarigina<sup>3</sup>, Ancha Baranova<sup>1,4</sup>, <sup>1</sup>School of Systems Biology, George Mason University, Fairfax, VA 22030, <sup>2</sup>Bioinformatics Institute, Agency for Science, Technology and Research, Singapore 138671, <sup>3</sup>K.1 Skryabin Academy of Veterinary Medicine and Biotechnology, Moscow 109472, Russia, <sup>4</sup>Moscow Institute of Physics and Technology, Moscow Region, Dolgoprudny 141700, Russia. Prenylation is a lipid

posttranslational modification of *de novo* synthesized proteins, when a farnesyl or gernylgeranyl group is added to specific site on C-terminal end of a protein. Prenylation of mammalian proteins plays an important role in different processes, such as cell proliferation, differentiation, apoptosis and general metabolism. Recent studies suggest that intracellular microorganisms can use eukaryotic prenylation machine for lipidation of its own proteins. This has been experimentally showed for S. *typhimurium* protein SifA, *L. pneumophila* protein AnkB, and hepatitis delta virus large antigen. In this study we perform analysis of all viral proteins that may be prenylated by human cells. Designed databases consist of human viral proteins both predicted from genome and experimental protein sequences from UniProt and NCBDI protein databases. Prenylation prediction suite (PrePS) predicted 35 viral proteins to be recognized by farnesyltransferase and 9 proteins by geranylgeranyltransferase 2. Interestingly, the vast majority of all identified prenylation events were found in RNA rather than DNA viral proteins.

NKG2D RECEPTOR COSTIMULATION DIFFERENTIALLY ACTIVATES SIGNAL TRANSDUCTION PATHWAYS DOWNSTREAM OF AKT IN MURINE EFFECTOR CD8 T CELLS. Kelsey M. Trace & Amorette Barber, Dept. of Biol. and Env. Sci., Longwood Univ., Farmville VA 23909. Effector CD8 T cells have potent anti-viral and anti-tumor functions. In order to become activated, naive CD8 T cells must be activated through the T cell receptor along with costimulation through the CD28 receptor. After activation, costimulation is no longer required but can greatly affect CD8 T cell functions, One costimulatory receptor that is likely to be stimulated on activated CD8 T cells is NKG2D because the ligands for this receptor are upregulated upon DNA damage, cells stress, and viral infection. Costimulation with NKG2D or CD28 has been shown to activate Akt, however it is unclear what signal transduction pathways downstream of Akt are activated by these receptors. Murine effector CD8 T cells were stimulated through the T cell receptor with and without NKG2D or CD28 costimulation and the activation of Akt and downstream pathways were analyzed using an In-Cell ELISA and RT-PCR, Compared to CD28 costimulation, stimulation through the T cell receptor and NKG2D lead to an increased phosphorylation of AKT and GSK-3β. Gene expression of members of the β-catenin, mTOR, and other pathways was also increased after NKG2D receptor stimulation. These data show that NKG2D costimulation of activated CD8 T cells could potentially enhance their anti-tumor and anti-viral effector functions. (Supported by: Longwood University's Faculty Research Grants and BES Department).

THE USE OF THAWED, ISOLATED LIVER CELL CYTOPLASM AS A NUCLEAR IMPORT MEDIUM. Jeffrey Branson, Haris Mirzada & Stephen Gallik, Ph. D., University of Mary Washington. Fluorescnee microscopy studies have shown that proteins lacking a nuclear localization signal (NLS) are excluded from the nucleus. These studies have shown that the fluorescence emission of tetramethylrhodamine isothiocyanate (TRITC)-labeled serum album (BSA) is never detected in nuclei when the labeled protein is incubated with isolated nuclei from various sources. This contradicts the results of autoradiographic studies that clearly show proteins the size of bovine serum albumin can penetrate the nuclear envelope and enter the nucleus very slowly through what is likely a simple diffusion process. This contradiction suggests

that the TRITC fluorophore may not be sensitive enough to detect the low concentrations of albumin that diffuse into the nucleus. The specific objective of this preliminary study is to assess the effectiveness of Alexa Fluor 488 fluorophore-labeled BSA in detecting low concentrations of BSA in the nucleus during its diffusion into the nucleus. Crude homogenates of rat liver cells were incubated for 45 minutes with Alexa Fluor 488-labeled histone H1, which contains an NLS, and Alexa Fluor 488-labeled BSA, which lacks an NLS. The results showed that Alexa Fluor 488 is sensitive enough to detect the very high concentrations of histone H1 that accumulated in nuclei as the result of an NLS-dependent nuclear import process as well as the very low concentrations of BSA that slowly accumulate in the nuclei as the result of simple diffusion.

HOST PLANT PHENOLIC ACIDS: OVIPOSITIONAL STIMULANTS OR DETERRENTS FOR CABBAGE WHITE BUTTERFLY, PIERIS RAPAE? Rebecca E. Dey, Skyler T. Carpenter, & Mary E. Lehman, Dept. of Biological and Environmental Sciences, Longwood University, Farmville, VA 23909. Cabbage white butterflics, Pieris rapae, lay their eggs on plants of the Brassicaccae family. Females may identify suitable hosts through the detection of various chemicals produced by the plants. Of particular interest are phenolic acids because they are found in high concentrations in Brassicaceae plants, yet little is known about the role they play in ovipositional choices. This study evaluated the potential of four phenolic acids to serve as ovipositional stimulants or deterrents for Pieris rapae. The phenolic acids tested were sinapic, vanillic, caffeic, and cinnamic acids. Treatments of phenolic acids and deionized water controls were applied to cabbage disks that were equal in leaf surface area and color. Each treated disk was matched with a DI H20 control and placed within an experimental enclosure with a single mated female. After 24 hours, the number of eggs laid on each disk was observed and recorded. Caffeic and sinapic acids both acted as significant deterrents. Cinnamic acid acted as a significant stimulant, Vanillic acid had no significant effect on oviposition. The combined results of this study and previous research suggest that it may not be possible to make broad generalizations about the effects of phenolic acids on ovipositional decisions and underscores the importance of assessing individual chemicals.

ANTIBIOTIC DYNAMICS OF ESCHERICHIA COLI POPULATIONS IN A STREPTOMYCIN-TREATED MOUSE MODEL. Hannah J. Drown & Andrew J. Fabich, Ph.D., Dept. of Biology and Chemistry, Liberty University, Lynchburg VA 24515. Escherichia coli infect both the urinary and gastrointestinal tracts in mammals. However, we note that the time required for a population to acquire antibiotic resistance has never been evaluated. By assessing antibiotic resistance at specific time points, we hope to mark the rate of E. coli antibiotic resistance in vivo. A previous in vivo study of E. coli fitness measured dynamic changes in motility of several populations over two months, discovering that changes surfaced in the commensal strain of E. coli Nissle. We hypothesize that the Nissle strain of E. coli within the murine intestine will similarly express spontaneous mutations that produce resistance to antibiotics. In this study, we examined the ability of E. coli to gain antibiotic resistance to nalidixic acid, chloramphenicol, novobiocin/SDS, rifampicin, streptomycin, and tetracycline. Each antibiotic was selected to highlight a separate mechanism of action by which resistance

should develop. Individual colonies from mouse feees colonized with the Nissle strain of *E. coli* were screened for genes associated with nalidixic acid, chloramphenicol, novobiocin/SDS, rifampicin, streptomycin, and tetracycline resistance. We compared the wild type *E. coli* growth on each antibiotic to colonies collected on day 7 and day 30 post-infection. The chloramphenicol, novobiocin/SDS, rifampicin, and tetracycline showed no changes in expression after growing *in vivo* for 30 days. However, increased resistance was noted in both the nalidixic acid and streptomycin by day 7 post-infection.

A COMMUNITY ANALYSIS OF THE INTESTINAL MICROBIOTA WITH CITROBACTER RODENTIUM COLONIZATION AND PATHOGENESIS. Cassandra E. Black & Andrew J. Fabich, Dept. of Biol. & Chem., Liberty Univ., Lynchburg, VA 24515. Current research does not clearly define how the intestinal microbiota affect gastrointestinal health, including protection from pathogens. EHEC 0157:H7 cause severe sickness and is life-threatening, but is difficult to study in vivo. Citrobacter rodentium (CR) is used to model EHEC infection in mice, but the intestinal microbiota response during CR colonization is not currently well-defined. CR were observed in both conventional and streptomycin-treated mouse models, and microbial population abundances were quantified using genus-specific primers and quantitative PCR. The results indicate that CR colonize better in conventional mice than in streptomycintreated mice. Bacteroidales and Bifidobacteria are unaffected by CR colonization, and Clostridia and Lactobacilli fluctuate during CR colonization with Lactobacilli ultimately decreasing. These outcomes may lead to EHEC prevention and clinical care on a global scale.

EVALUATION OF ALTERED GENE EXPRESSION PATTERNS IN BRAIN AND BLOOD FOR AN ALZHEIMER'S DISEASE MOUSE MODEL. A. Házy, M. Baker, R. Haraf & G. D. Isaacs, Department of Biology and Chemistry, Liberty University, Lynchburg VA 24515. Gene expression patterns are known to affect the development and pathology of Alzheimer's disease (AD). To determine the specific changes in expression involved in AD, differential gene expression in brain and blood for an AD mouse model was determined using RNA sequencing. RNA was isolated from brain and blood of AD and control mice (n=3), reverse transcribed to eDNA, and sequenced to determine genome-wide expression. Sequence data was analyzed using the Galaxy platform to identify differentially expressed transcripts. The analysis found 5053 transcripts differentially expressed in the brain for an AD model, including multiple genes known to affect AD progression. Expression data was compared to previously determined changes in methylation and hydroxymethylation to evaluate epigenetic effects on gene expression. While many genes showed a definite increase or decrease in expression with epigenetic changes, other genes displayed splice variant specific changes in expression—certain isoforms of the same gene increased expression and others decreased. This data suggests that epigenetic changes may alter splice variant selection at specific genes. Expression patterns in blood were also determined for comparison to those found in the AD brain. (Supported by: the Jeffress Memorial Trust and the Alzheimer's and Related Diseases Research Award Fund).

A STUDY OF CHLOROQUINE'S ANTIRETROVIRAL CHARACTERISTICS. Justin E. P. Sahs and Lynn O. Lewis, Dept. of Biol. Sci., University of Mary Washington, Fredericksburg VA 22401. Mouse Mammary Tumor Virus, known as MMTV, is a retrovirus transmitted in mice through their breast milk which has been proven to lead to multiple different types of cancer, including breast, ovarian, and prostate cancer. Due to its resemblance to other retroviruses and a relative level of safety towards humans, MMTV is an ideal candidate to act as a research model for retroviruses such as HIV, making it highly relevant in the study of human medicine. The virus enters host cells through receptor mediated endocytosis, which is initiated by the acidification of the vesicle, Chloroquine, which is a weak base, acts as a buffer to minimize acidification and greatly the reduce infectivity of the virus. In research performed last year by UMW student Ryan Green, chloroquine was shown to reduce viral infectivity in MMTV-infected cell cultures most effectively at doses between 2.095E-04 and 2.095E-05 μM. The aim of this study was to prophylactically treat MMTV-infected cells with chloroquine, induce replication with the hormone dexamethasone, and then to isolate and visualize the viral RNA through a spin cycle followed by rtPCR techniques. We found that we were able to visualize the viral RNA via rtPCR followed by gel electrophoresis. Because the chloroquine-treated group was able to be visualized, we can conclude that chloroquine does not eliminate all viral reproduction in hormonally-stimulated MMTV-infected cells.

BIOINFORMATICS AND TRANSCRIPTION FACTORS IN ALZHEIMER'S DISEASE. John R. Davy, John Lawson & Gary D. Isaacs, Dept. of Biology, Liberty University, Lynchburg, VA. 24515. Alzheimer's Disease (AD) is characterized pathologically by neurofibrillary tangles and amyloid-beta plaques, but underlying these processes lie epigenetic changes. Specifically, methylation and hydroxymethylation of cytosine residues in gene promoters changes during the disease process. These changes, either an increase or a decrease in methylation or hydroxymethylation, modulate the binding of transcription factors to the promoter region. We hypothesize that the epigenetic alterations affect specific transcription factors, which alter the gene expression profile seen in AD, Bioinformatics allows high throughput of data, comparing the transcription factors enriched in genes affected by epigenetic changes and those altered in gene expression. Linking these two changing components in AD through TFs provides a dataset for further research into how these TFs are involved in the disease process. We found that a number of TFs, such as USF1, USF2, and ARNT are enriched in both genes changing drastically in epigenetic modifications and in genes changing in expression. Our study also demonstrated that TFs already linked to AD (such as AP1, USF1, and ARNT) are also enriched in epigenetically modified genes, showing the involvement of methylation and hydroxymethylation in the disease process. This work is supported by the Jeffress Memorial Trust (Grant J-998), the ARDRAF grant, and the Virginia Academy of Science.

VISCERAL ADIPOSE DEPENDENT INDUCTION OF AUTOIMMUNE RESPONSE. <u>Allison M. Heath</u>, Katherine Doyle, Aybike Birerdine & Ancha Baranova, School of Systems Biol., George Mason Univ., Fairfax VA 220304444. One of the numerous adverse effects of the accumulation of excess visceral adipose is that

resulting alteration of cytokine signaling. A state of morbid obesity results in the interference of the body's pro and anti-inflammatory pathways resulting in a systemic pro-inflammatory state and are regulated by such cytokines as IFN-g, TGF-b, TNF-a, IL-1, IL-4, IL-10, IL-12 and IL-8, regulate TH1 and TH2 pathways, which are associated with immune and inflammatory responses. Alteration of the normal function of these pathways may be linked to the development of autoimmune disorders in the morbidly obese. For example in animal models of multiple sclerosis, mice with defective TRIF or type I IFN receptors developed more severe experimental allergic encephalomyelitis than mice with normally functioning receptors and showed an increase in IL-17 production. It has also been shown that embedded in visceral adipose tissue are CD4+ T lymphocytes that govern insulin resistance in mice with diet-induced obesity. Given the role of these proinflammatory regulatory cytokines in both obesity and autoimmune diseases, it appears that the excessive accumulation of visceral adipose tissue may trigger a cascade of events that induces various manifestations of autoimmune diseases particularly in morbidly obese patients.

OUERCETIN AS AN ANTI-INFLAMMATORY AGENT RELEVANT TO THE MANAGEMENT OF METABOLIC SYNDROME. Anna Zhang<sup>2</sup>, Ancha Baranova<sup>1,2</sup>, Aybike Birerdinc<sup>1,2</sup>, <sup>1</sup>Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church VA and <sup>2</sup>Center for the Study of Chronic Metabolic Diseases, School of Systems Biology, George Mason University, Fairfax, VA. Obesity-associated deaths have risen to more than 300,000 a year in the United States, and have been reported as having the second highest mortality rate, second to only tobacco-related deaths. An extensive list of morbidities, such as Metabolic Syndrome (MS), cardiovascular disease (CVD), diabetes mellitus type II (DMII), stroke and cancer is heavily linked to obesity. Complications from obesity and its comorbidities are what makes obesity such a great financial burden. Extensive research has implicated chronic-low grade inflammation induced by dysfunctional adipocytes as a key associate of Mctabolic Syndrome, a term coined to cluster major risk factors that are etiologically relevant to the development of cardiovascular disease (CVD) and type II diabetes mellitus (DM), including abdominal obesity, atherogenetic dyslipidemia, hypertension, glucose intolerance. Quercetin, a flavanol ubiquitous in fruit, tea, wine and stems and leaves of vegetables, have been shown to exert an anti-inflammatory action by scavenging adipocytic secretions of pro-inflammatory cytokines from macrophages, activating peroxisome proliferator-activated receptors (PPAR)-gamma, and thus suppressing the activation of pro-inflammatory transcription factor nuclear factor-kappa (NF-κB), and enhancing expression of anti-inflammatory cytokines. The aim of this study is to assess the potential health benefits of quercetin as it relates to the management of metabolic syndrome via its anti-inflammatory mechanisms.

THE EXPRESSION OF KCTD FAMILY GENES IN ADIPOSE TISSUE OF PATIENTS WITH DIET INDUCED OBESITY. Kuan Yao<sup>1,2</sup>, Kataline Anderson<sup>1,2</sup>, Aybike Birerdinc<sup>1,2</sup>, & Ancha Baranova<sup>1,2</sup>, <sup>1</sup>Betty and Guy Bcatty Center for Integrated Research, IHS, Falls Church VA 22042, <sup>2</sup>Center for the Study of Genomics in Liver Discases, Molecular and Microbiology Department, GMU, Fairfax, VA. Potassium Channel KCTD proteins have been suggested in the development of diet induced obesity. KCTD interferes with the normal assembly of the voltage-gated potassium

channels causing the suppression of the voltage-gated channels. Diet-induced obesity was shown to impair endothelium-derived hyperpolarization via altered K channel signaling. Previous studies of KCTD15 have revealed the repression of AP-2α, which inhibits the activity of C/EBPα, an early inducer of adipogenesis. However there is little data on the levels of KCTD15 and other KCTD proteins in the visceral adipose (VAT) of morbidly obese patients. The aim of this research is to determine the relative expression levels of KCTD family genes, such as KCTD1, 9, 12, 15, and 18 in the VAT of a cohort of obese and morbidly obese patients. Currently 12 adipose samples had been extracted and analyzed using qRT-PCR. The delta-delta-ct method was used to investigate the relative levels KCTDs in samples. All KCTD genes have shown a decrease in expression in non-NAFLD samples, However, an increasing trend of gene expressions was observed in KCTD9, KCTD12, and KCTD15 as the levels of NAFLD increase. KCTD1 and KCTD18 gene expressions were variable due to diversity in patients' age, BMI, disease state, and other factors. The assessment of several KCTD family of proteins in the visceral adipose of morbidly obese patients will shed some light on their involvement in the etiology of obesity and its comorbidities.

THE OBESITY LINKED IMMUNE FUNCTION. Momina Tariq, Ancha Baranova<sup>1,2</sup> and Aybike Birerdinc<sup>1,2</sup>, <sup>1</sup>Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church, VA, <sup>2</sup>Center for the Study of Chronic Metabolic Diseases, George Mason University, Fairfax, VA. Immune and metabolic systems are among the most primary requirements for survival of a human being. Research in the field of metabolic diseases over the last decade have demonstrated a clear association between weight regulation and inflammation. The immune response and metabolic regulation are highly integrated and the proper function of each is reliant on the other. Dysfunction of one can lead to numerous chronic metabolic disorders, diabetes, fatty liver and cardiovascular diseases. The aim of this present systematic review is to explain the detrimental effects of obesity on the immune system and how it spearheads towards other immune-allied complications. A systematic PubMed literature search was designed. Studies were included that explained the inflammation of adipose tissue as an important protagonist in the development of obesity related problems and studies that interconnected the link between obesity-immune system and systemic inflammation. These preliminary findings indicate that obesity leads to the change in the number of leucocyte count, which instigates impaired immunocompetence and may result in a weakened immune system. Inflammation is characterized by changes in immune cell populations giving rise to altered adipo/cytokine profiles, which in turn induces/stimulates insulin resistance and other diseases such as NAFLD and NASH.

GRIFFONIA SIMPLICIFOLIA ISOLECTIN I-B4 AS A MOLECULAR TAG FOR IMMUNOGLOBULIN. P. Tedrick, E. Marakasova & A. Baranova, School of Systems Biology, George Mason University, Fairfax VA 22030-4444. Lectins are proteins that have high specificity for carbohydrates and can bind reversibly with them by carbonate recognition domains. The isolectin I-B4 (GSI-B4) from Griffonia simplicifolia has a marked specificity for a non-reducing terminal μ-D-galactose. We hypothesized that GSI-B4 carbonate recognition domain will bind to immunoglobulin G, because it has an N-glycosylation site that would allow the binding. Therefore an ELISA-like single step assay could potentially be developed to test the binding of GSI-B4 to

immunoglobulins. Partial gene GSI-B4 was synthesized and used for site specific cloning in pQE-30 and pQE-RFP plasmids. Cloning used two restriction enzymes BamHI and SacI for pQE-30, and KpnI and HindIII for pQE-RFP plasmid. T4 DNA ligation reactions were transformed to *E. coli* DH5µ, and positive samples were verified by DNA sequencing. Expression in *E. coli* BL-21 shows at low levels at 10-hour time point after IPTG induction. Expression does not increase substantially by the 24-hour time point. After optimization of expression and verification by Western Blotting, next steps will be to purify GSI-B4. This purified product will be used to develop an ELISA-like assay to test binding of GSI-B4 to immunoglobulin.

A COMPARISON OF GENE EXPRESSION IN RESPONSE TO BPA EXPOSURE IN PROSTATE AND MAMMARY TISSUES. Daniel E. Miller & Deborah A. O'Dell, Dept. of Biol. Sei., Univ. Mary Washington, Fredericksburg, VA 22401. Bisphenol A (BPA) is a synthetic carbon-based compound that has been shown to exhibit hormone-like properties in its ability to act as a ligand to estrogen and androgen receptors. This study aims to investigate similarities in the transcription-altering pathways of BPA in mammary and prostate cells with regards to oncogenes and tumor suppressor genes in untreated control and BPA-treated experimental groups. Previously extracted RNA from human mammary and prostate cells was assessed for integrity and reverse-transcribed to cDNA via PCR. Genomic profiles were obtained through microarray analysis (Oncogenes and Tumor Suppressor Genes PCR Array, SA Bioscience) and analyzed for fold-change in expression from control using software supplied with the assay. Using a threshold fold-change of ± 2.00, BPA exposure resulted in primarily opposite responses in gene expression between the cell lines (i.e. genes up-regulated by BPA in the mammary group tended to be down-regulated in the prostate group). However, there were two oncogenes (JAK2; MYCN) that were upregulated and two tumor suppressor genes (BRCA1; TP53) that were down-regulated in response to BPA exposure in both mammary and prostate cells. Although JAK2 and MYCN have not been directly linked to mammary or prostate cancers, mutation in both the BRCA1 and TP53 genes is known to be implicated in breast, ovarian, fallopian tube, and prostate cancers, as they play an important role in DNA repair in these cell types. These results suggest a mechanism for the conversion of mammary and prostate epithelial cells to the cancerous state.

CELL PHONE RADIATION INDUCED GENE EXPRESSION IN HUMAN CLIOBLASTOMA CELLS. V. L. King, D. Delfino & D. A. O'Dell, Dept. of Biol. Sci., Univ. Mary Washington, Fredericksburg, VA 22401. The average American spends 25 minutes a day talking on a cell phone. Cell phones emit electromagnetic radiation that ranges from 106 to 1012 Hertz, which has been shown to produce free radicals that can induce changes in gene transcription. This study examined gene expression changes in human glioblastoma cells over time after cell phone radiation exposure. Human glioblastoma cells were cultured and when confluent were exposed to 25 minutes of cell phone radiation. The cells were lysed and the RNA was extracted immediately, 20 minutes, 24 hours, and 48 hours after exposure. The RNA was converted to cDNA and placed on a commercial RT PCR 96 well plate containing the primers for 84 oncogene and tumor suppressor genes (SABiosciences Corporation: Frederick, MD). RT PCR was performed using a Stratagene 3005p Thermocycler, and

the data was analyzed using the SABioscience PCR Array Data Analysis tool. The results showed that the effects of radiation on gene expression changed as time post-exposure increased. Cell cycle regulation was altered by exposure temporarily allowing increased proliferation that was then reversed. Alteration in genes promoting the cancerous state were up-regulated at 24 hours and then the expression was almost reversed by 48 hours post-exposure. These results provide implications for how constant exposure to cell phones can lead to cellular changes in susceptible cells potentially producing cancerous conditions. Future studies may incorporate larger sample sizes as well as increased and decreased time after exposure for RNA extraction such as 36 and 72 hours after exposure.

COMPARATIVE ANALYSIS OF LUXR REGULATION OF THE QUORUM SENSING IN MESOPHILIC AND PSYCHROPHILIC BACTERIA. Manukhov<sup>1,3</sup>, Ancha Baranova<sup>1,2</sup> & Svetlana Khrulnova<sup>3</sup>, <sup>1</sup>Department of General Physics, Moscow Institute of Physics and Technology (State University), 9 Institutsky Lane, Dolgoprudny, 141707, Russia, <sup>2</sup>School of Systems Biology, George Mason University, Fairfax, VA, 3State Research Institute of Genetics & Selection of Industrial Microorganisms (GosNIIGcnetika), Moscow, Russia. Luminescent psycrophiic marine bacteria Aliivibrio logei are assumed to be widely distributed in cold waters of northern seas. Here we characterize the structure features of lux operon of psychrophilic KCh1 and BM1 strains of the marine luminescent bacteria A. logei isolated from the intestines of goby fish indigenous to the White Sea and Sea of Okhotsk, Kamchatka. The A. logei lux operon was cloned in Escherichia coli cells and the structure of this operon and its nucleotide sequence were determined. In the structure of the A, logei lux operon, the the luxI gene is absent in front of luxC, and a fragment containing luxR2-luxI genes is located immediately after luxG gene. Thus, it resembles the lux operon of Aliivibrio salmonicida. We compared bioluminescent characteristics of this operon with that of mesophilic marine luminescent bacteria Aliivibrio fischeri. In spite of structural differences, these two operons exhibited similar sensitivities to autoinducer.

REMOVAL OF OXIDIZED CIRCULATING DNA MAY AUGMENT EFFECTS OF CANCER THERAPY. A. Baranova<sup>1,2</sup>, S. V. Kostyuk<sup>1</sup>, T. D. Smirnova<sup>1</sup>, L. A. Kameneval & N. N. Veikol, Research Centre for Medical Genetics, Russian Academy of Medical Sciences, Moscow, <sup>2</sup>Center for the Study of Chronic Metabolic Diseases, School of System Biology, George Mason University, Fairfax, VA. Cell-free DNA fragments (cfDNA) are biologically active as they are enriched in 8-oxo-dG. Short-term exposure to oxidized DNA results in both single- and double strand DNA breaks. Longer treatments evoke a compensatory (adaptive) response. In breast carcinoma model MCF-7, an exposure to oxidized extracellular DNA stimulates the survival and an increase in genome instability. Hence, the oxidized DNA is a stress signal released in response to oxidative stress in the cultured cells and, possibly, in the human body; in particular, it might contribute to systemic abscopal effects of localized irradiation treatments. The mass release of oxidized DNA that accompanies apoptotic and necrotic processes in radio- and chemotherapy treated tumors may aid survival of residual cancer cells and even instigate their resistance to further treatment. It is likely that the selective antibody-guided removal of oxidized DNA from the bloodstream or the block

of respective oxidized DNA-dependent signaling may be developed as an adjuvant treatment for antitumoral therapy.

GLOBAL VARIATIONS IN HBV ENDEMICITY, VACCINE COVERAGE, AND GENOTYPE DISTRIBUTION. Max Marzouk, Ancha Baranova & Aybike Birerdinc, College of Science, George Mason Univ., Fairfax VA. 22030-4444. Despite the advent of an effective vaccination in the 1980's against the hepatitis B virus (HBV), chronic HBV infection remains the world's leading viral cause of hepatocellular carcinoma (HCC). Based on a nucleotide divergence greater than 8%, HBV can be classified into 10 genotypes (A-J) and several subgenotypes with distinct geographic distributions. Natural history studies have indicated that genotype influences clinical outcome and interestingly, the genotypes associated with more severe liver disease predominate in regions with relatively high hepatitis B surface antigen (HBsAg) scroprevalence - an indicator of HBV infection. Here we attempt to characterize global variations in HBV genotype distribution, endemicity, and vaccine coverage - so that emerging nonsynchronous trends can be used to identify regions where HBV resource and education allocation should be prioritized. Recent studies and joint reports by the World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) were the primary sources selected for the analysis of HBV vaccine coverage. Genotyping studies were systematically selected from references in highly cited and relevant publications and Pubmed/Medline keyword searches. Regional seroprevalence and vaccine coverage levels were clustered, mapped, and evaluated. West, East, and Southern Sub-Saharan Africa consistently reported high HBV vaccine coverage during periods of very high endemicity. Similar trends were identified in Central and East Asia. The predominant genotypes in these regions are B, C, and E.

AN ASSOCIATION BETWEEN THE DEPRESSION AND SYSTEMIC INFLAMMATION IN NHANES POPULATIONS. Rachel Smith, Maria Stepanova, Ancha Baranova, & Aybike Birerdinc, Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church VA and Center for the Study of Chronic Metabolic Diseases, George Mason University, Fairfax, VA. A population-based study was conducted to determine the association of individuals with chronic diseases and depression from five cycles of the National Health and Nutrition Examination Survey (NHANES) data. The presence of depression was determined from a depression screener questionnaire, while the presence of chronic diseases were gathered from medical conditions questionnaires (3,751 and 26,225 subjects respectfully). Interestingly, individuals with depression and those with multi-morbidity had the same statistically significant increased inflammatory markers as assessed by HOMA scores, C-reactive proteins, white blood cell counts, and percentages of segmented neutrophils and glycohemoglobin (p<0.05). Additionally, both had statistically significant decreased levels of HDL-cholesterol, lymphocyte percentages, and monocyte percentages (p<0.05). The increased inflammatory markers suggest that low-grade chronic inflammation may be the connection between depression and chronic diseases in individuals. Examining these inflammatory markers may be used as predictors in the treatment of patients.

NKG2D RECEPTOR ACTIVATION OF NF-KB ENHANCES INFLAMMATORY CYTOKINE PRODUCTION IN MURINE EFFECTOR CD8+ T CELLS. Emily Whitman & Amorette Barber, Dept. of Biol. and Env. Sci., Longwood Univ., Farmville VA 23901. To induce strong immune responses, naïve CD8<sup>+</sup> T cells require stimulation through the TCR and costimulatory receptors. However, the effect of activating costimulatory receptors on activated T cells is unclear. The NKG2D costimulatory receptor is expressed on CD8<sup>+</sup> T cells with its ligands expressed on tumor cells and during some infections. In order to determine how activation of costimulatory receptors alters effector CD8<sup>+</sup> T cell functions, this study compared the activation of the NF-κB signaling pathway by two costimulatory receptors, CD28 and NKG2D, Activation of murine effector CD8+ T cells through CD3 and NKG2D receptors enhanced activation of NF-kB as shown by increased phosphorylation of IKK $\alpha$ , IkB $\alpha$ , and NF-kB and IkB $\alpha$  degradation. Activation of the NF-kB pathway lead to increased secretion of pro-inflammatory cytokines, including IFN-alpha and IFNgamma, and decreased secretion of anti-inflammatory cytokines, including IL-10 and CCL2. NF-кВ activation also increased the effector molecules TNF-alpha, lymphotoxin alpha and beta, and Fas ligand. These data show that stimulation through NKG2D leads to the differential activation of signaling pathways and potentially enhances the antitumor and anti-viral functions of effector CD8<sup>+</sup> T cells. (Supported by: Longwood University's PRISM program, Faculty Research Grants, and BES department).

#### Biomedical and General Engineering

STORMWATER RUNOFF FILTRATION MEDIA FOR TRACE METAL CAPTURING. Gail M Moruza, Zach Goehring & Brittany Toney, James Madison Univ., Harrisonburg VA, 22801. Nationwide, stormwater runoff has been identified as the leading cause of water-quality impairment due to the trace metals found within that accumulate over time and create potentially toxic chemical imbalances in ecosystems. Effective stormwater runoff filtration is a key part in preventing dangerous contaminants from being released into the environment and causing harm to the health of aquatic and terrestrial organisms and eventually humans. In particular, the accumulation of trace metals in coosystems and eventually human drinking water has been known to cause cancer, high blood pressure, liver disease, kidney disease, growth reduction, growth abnormalities, and chronic anemia in the organisms which ingest the metals. The hazardous amassing of trace metals can be alleviated through the study of the most effective filtration media such as zeolite, perlite, and peat. Essential factors affecting filtration efficacy, which are covered in this study, include mass of media and media type used, which in turn determines the mechanisms by which contaminants are captured from solution. From the initial data of this research, it was determined using a one-way ANOVA procedure that no significant difference in filtration capabilities existed between the media types examined. The ANOVA procedure did, however, determine that a greater media mass captured a significantly greater amount of trace metal from solution than did the smaller media mass.

EX VIVO DNA ASSEMBLY. <u>Zachary B. Canfield</u>, Adam B. Fisher & Stephen S. Fong, Department of Chemical and Life Science Engineering, Virginia Commonwealth

University, Richmond VA 23284-2006. Our ability to re-design existing natural biological systems for useful purposes is limited by our capability to design and build DNA sequences that encode specific functionality. However, it is still a challenge to engineer biology in a directed manner through logical genetic design. To circumvent this problem synthetic biologists build large libraries of genetic parts where - by rigorous characterization – sequence design principles and suitable biological solutions ascertained. Unfortunately, de novo DNA synthesis is still cost prohibitive, especially when testing combinatorial libraries, so it is more cost effective to purchase DNA as short "building blocks" and assemble them in-house. While powerful methods exist for DNA assembly, current approaches suffer from trade-offs of being either timeconsuming or monetarily expensive. The ex vivo DNA assembly method is fast and efficient at assembling DNA while remaining relatively inexpensive in that they only require lysate harvested from a common laboratory strain of Escherichia coli, This makes the ex vivo cloning approach particularly attractive in experimental schemes where large libraries of constructs must be created and screened for desired functionalities.

FIBRONECTIN MECHANICS AND SIGNALING IN EPITHELIAL TO MESENCHYMAL TRANSITION. Lauren A. Griggs, Min Zhao, Roshni Malik, Dr. Lynne Elmore, & Dr. Christopher Lemmon, Dept. of Biomed. Engr., Virginia Commonwealth Univ., Richmond VA 23284-3067. This work is part of an ongoing study that investigates the role of the microenvironment in the induction of Epithelial to Mesenchymal Transition (EMT). Previous studies have shown that Transforming Growth Factor-bcta-1 (TGFβ-1) drives epithelial cells to undergo EMT; however, the precise mechanism of TGFβ-1-induced EMT is not well understood. Our research examines the relationship between assembly of the extracellular matrix protein fibronectin (FN) into insoluble fibrils and the occurrence of EMT. Coupled with evidence that increased tissue stiffness is a modulator of disease severity, we have crafted the following over-arching hypothesis: increased tissue stiffness drives FN assembly, which exposes cryptic binding sites for various growth factors, such as TGF\$\beta-1, and creates a high concentration of these growth factors at the cell surface, which in turn drives EMT. The current work examined the role of FN assembly in nontransformed, healthy MCF10A mammary epithelial cells, Results showed that nontreated epithelial cells assembled very little FN and maintained high levels of epithelial markers, while TGFβ-1-treated cells had a marked increase in FN assembly, as well as increased mesenchymal markers and decreased epithelial markers. Inhibition of FN fibril assembly was shown to inhibit EMT progression. By establishing an innovative mechanism by which growth factor signaling induces EMT through interaction with the ECM, this research will serve to combat the development and initiation of metastasis, and potentially identify novel targets within the tumor microenvironment for cancer therapy.

BUILDING 3D TISSUE UTILIZING MECHANICALLY STIMULATED SPATIAL ORGANIZATION AND DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS. <u>Jiten D. Narang</u> & Christopher A. Lemmon, Dept. of Biomedical Engineering, Virginia Commonwealth University, Richmond VA 23284. Our research utilizes an array of deformable microfabricated pillars to provide mechanical stimuli

to control alignment and spatial organization of cells while also influencing differentiation. Manipulating cross sectional area and geometry as well as the height of the pillars provides precise control of the spatially-localized substrate stiffness. Varying stiffness in different regions allows us to direct differentiation into multiple lineages within one cell monolayer. A novel stacking method is used to stack these sheets, developing a 3D tissue with controlled architecture. Human mesenchymal stem cells are plated on top of the pillars with the cleavable fibronectin facilitating the attachments between cell and substrate. Providing cells with mechanical cues from the oval pillars influences cellular and ECM spatial organization. Once confluent and organized, cell layers are treated with a blend of osteogenic and adipogenic induction medias that complement the respective mechanical cues to induce differentiation. This organized and differentiated cell layer can then be inverted, brought into contact with the assembling 3D microtissuc, and disassociated from the pillars. Cells adhere to the growing microtissue and arc left behind once the inverted pillar substrate is removed. Multiple repetitions of this method yield a structurally organized, cell type specific 3D tissue. Results show that providing a stiffness gradient allows for accurate spatial alignment and organization of cells on microfabricated pillar arrays.

EQUILIBRIA AND NO-REGRET STRATEGIES IN CANCER TREATMENT. Natasha D. Sheybani<sup>1</sup>, Ishaan A. Nerurkar<sup>2</sup>, & Hu Yang<sup>1</sup>, <sup>1</sup>Department of Biomedical Engineering, Virginia Commonwealth University, Richmond VA 23298 and <sup>2</sup>School of Engineering and Applied Sciences, University of Pennsylvania, Philadelphia PA 19104, In Orlando et al. 2012, Cancer Treatment as A Game: Integrating Evolutionary Game Theory Into the Optimal Control of Chemotherapy, the authors propose a model in which the tumor's strategy is determined by natural selection and the oncologist's strategy is determined by the solution to a control problem. In this paper, we adopt the evolutionary growth model from Orlando et al., and define payoff functions for the oncologist and the tumor cell to model the interaction as a zero sum game. John von Neuman's minmax theorem proves that for every finite two player zero sum game, the security strategies of the players are equilibrium strategies. We apply this to define the equilibrium strategies of the game, which by evolutionary logistic growth equations and the payoff functions of the game formulated in this paper, predict the population size of the tumor. We then extend this model to a dynamic setting, in which the oncologist administers a drug over a defined treatment period. We propose a weighted majority algorithm which recommends drug delivery rates at every administration for the oncologist. The algorithm has a no regret property, which ensures that by the end of the treatment period, the average loss from the rates chosen by the oncologist is asymptotically no worse than if the oncologist had known the best fixed delivery rates in hindsight. This study holds vast applications to the optimized engineering and administration of drug cocktails for chemotherapeutic applications.

EYE TRACKING: A NOVEL TOOL FOR OBJECTIVE ASSESSMENT OF mTBI. Jefferson W. Overlin, Cassie P. Turnage, Ankita Dosaj, Kristina M. Kelly, & Paul A. Wetzel, Ph.D., Department of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA 23284. Mild traumatic brain injury (mTBI) is common among active duty military personnel, civilians in the workplace, and athletes competing in contact sports. The effects of mTBI on mental capability and stamina

have been widely documented. However, objective assessment of mTBI can be challenging. The measurement of eye movements using current eye tracking technologies represents a novel, efficient, and objective means of detection of mTBI. Visual stimuli to evaluate functional eye movement capability were developed for active duty military personnel diagnosed with mTBI and a control group. Eye movement data were collected with two-dimensional eye tracking systems at 500 Hz (Eye Link II, SR Research) and 1000 Hz (Eye Link 1000, SR Research) in two separate ongoing studies. Data were analyzed for response latency, number of saceades, eye movement amplitude, duration, velocity, acceleration, accuracy, and stability. To date, we have found significant differences for several eye movement tasks and parameters during reading, saceadic tracking, and smooth pursuit tracking, suggesting that the areas of the brain associated with these eye movements are sensitive to mTBI.

CONTROLLED RELEASE OF RAT-ADIPOSE DERIVED STEM CELLS FROM ALGINATE MICROBEADS. S. K. Leslie D. J. Cohen, B. D. Boyan, Z. Schwartz <sup>2</sup> Dept. of Chemical and Life Science Engineering and Dept. of Biomedical Engineering, VA Commonwealth University. One notable disadvantage of cell based therapies is that the injected cells tend to disperse away from the intended site. The aim of this project is to develop a system of injectable hydrogels to deliver stem eells for the purpose of tissue regeneration, thereby allowing the cells to remain at the area of injury, to proliferate and secrete soluble factors that will facilitate tissue regeneration. The hydrogel employed is alginate; however; it does not readily degrade in vivo for six months. We demonstrated the controlled release of viable cells from degradable alginate microbeads via alginate-lyase mediated degradation in vitro. Degradable alginate microbeads were made by combining equal volumes of LVM alginate and alginate-lyase solution to form alginate microbeads of various ratios of alginate-lyase to alginate (1.75 U/g to 0.06 U/g). The results showed that cells released from the degradable microbeads maintained their osteogenic phenotype. In addition we showed that microbcads were able to degrade in vivo and their by-products did not illicit an inflammatory response. Lastly, we demonstrated the ability of the degradable alginate microbeads to localize cells at the delivery site in vivo. These results indicate that the system of degradable hydrogels can localize and release viable cells at the site of interest.

EVALUATION OF A NOVEL BLOOD ANALOG FOR MOCK CIRCULATION TESTING. Stephen J. Warren, Gerald E. Miller., Biomedical Engineering Department, Virginia Commonwealth University, Richmond VA 23284. For this experiment the object is to look into the effects of shear dependent viscosity on the flow fields with a physiological model. To isolate the parameters of the experiments the flow that is set to be investigated is the flow of blood out of the aortic valve into the aortic root. This is an area of flow which non uniform shear rates which should have a pronounced effect on the velocity profile of a Newtonian versus non Newtonian fluid. The focus here is the comparison of shear dependent fluids and the fact that the fluid is tunable and controlled based on its computational model and the flow apparatus, the aorta, is made from open source patient-specific data that can be used to recreate that specific aorta. The valve for these experiments will be a tilting disc valve, which allows for known flow characteristics.

CARPAL KINEMATICS IN THE DURING PREDICTING WRIST SCAPHOLUNATE LIGAMENT DISSOCIATION. Edward J. Tremols & Dr. Jennifer S. Wayne, Orthopedic Research Lab, Depts. of Biomedical Engineering & Orthopedic Surgery, Virginia Commonwealth Univ., Richmond VA, 23284. The wrist is a complex structure consisting of articulations between multiple bones. The carpal bones are found between the radioulnar articulation and the metacarpals and provide stability to the wrist. Injuries are common with varying success of surgical reparative procedures. This study proposes a computational model to predict scapholunate carpal kinematics throughout wrist motion where biomechanical function is determined by accurate 3D articular anatomy, ligament tensions, muscle forces, and external loading. A rigid body model was created using a high resolution CT scan of a male left arm. The set of CT images was brought into MIMICS, and accurate 3D bony anatomy created. These solid bodies were assembled in SolidWorks/Motion and force elements were applied to replicate the physiologic loadings experienced through tendons and ligaments. Validation was accomplished via comparison to a previous cadaver study which sought to understand scapholunate kinematics with the scapholunate intact and excised. Agreement between model and experiment was very good to excellent. The model also has the capability of predicting other important biomechanical parameters that are not typically feasible experimentally such as joint contact forces and ligament tensions. These will be explored for surgical procedures to restore scapholunate function.

LASER SINTERED TITANIUM SURFACES MIMIC TRABECULAR BONE STRUCTURE AND INDUCE OSTEOBLAST DIFFERENTIATION IN A POROSITY-DEPENDENT MANNER. A. Cheng<sup>1</sup>, D. J. Cohen<sup>2</sup>, S. L. Hyzy<sup>2</sup>, A. Humayun<sup>2</sup>, B. D. Boyan<sup>3</sup> & Z. Schwartz<sup>3</sup>. Dept. of Biomedical Engineering, GA Institute of Technology, Emory University & Peking University, <sup>2</sup>Dept. of Biomedical Engineering, VA Commonwealth University, and <sup>3</sup>School of Engineering, VA Commonwealth University. An increasing number of orthopaedic and dental implants are being implanted and must successfully serve a longer lifespan. Selective laser sintering (SLS) is a form of additive manufacturing that can produce customized, porous titanium alloy implants with high resolution. We created 3D titanium surfaces with low, medium and high porosity based on a human trabecular bone template, which were further blasted and pickled to induce combined micro-/nano-roughness. We characterized the porosity (41-76%), surface chemistry (Ti, O and C being the three most prominent elements), wettability (62  $\pm$  18 degrees), roughness and topography (showing combined micro-/nano-roughness). We then analyzed MG63 osteoblast response to surfaces. Cell viability on surfaces with varying porosity was not significantly different. DNA (proliferation) and alkaline phosphatase specific activity (carly osteoblast differentiation) decreased with increasing porosity, and osteocalcin (late osteoblast differentiation), osteoprotegerin (bone remodeling), VEGF (blood vessel formation), and BMP2, 4 (factors for creating an osteogenic environment) increased with increasing porosity. The most favorable cell response was shown on high porosity surfaces. The results of the present study indicate that surface modification and three dimensional structure can enhance bone apposition and osseointegration.

A LOW COST HAPTIC DISPLAY FOR VIRTUAL SIMULATIONS IN A 3D ENVIRONMENT INTENDED FOR USERS WHO ARE BLIND OR VISUALLY IMPAIRED. R. Petrella<sup>1</sup>, D. Pawluk<sup>1</sup> & N. Guidice<sup>2</sup>, <sup>1</sup> Department of Biomedical Engineering, Virginia Commonwealth University, Richmond VA 23284 and <sup>2</sup>Department of Spatial Information Science and Engineering, University of Maine, Orono Maine 04469. Individuals who are blind or visually impaired lack access to rich and immersive graphical and pictorial diagrams. Presented is a tactile display that allows the user to explore and interact with 3D, virtual diagrams. The device combines four elements: a computer/laptop, LeapMotion (motion tracking), LabView Program, and piezoelectric elements (viobrotactile feedback), By outputting vibratory frequency to the user, information about the size, direction, distance, and location of the virtual object can be communicated. Additionally, changing the parameters of texture, feedback pattern, location of feedback (finger, hand, or tool), or number of points of contact can enrich the user experience. Preliminary testing shows the ability of the device to act as a basic user interface in a 3D space in real time. The authors are interested in continuing this research with a human trial. This study was generously funded by NSF Award #1218310.

TITANIUM SURFACE PROPERTIES ALTER MACROPHAGE ACTIVATION. Kelly Hotchkiss, Sharon Hyzy, Zvi Schwartz, Barbara Boyan & Rene Olivares-Navarrete, <sup>1</sup>School of Engineering, Virginia Commonwealth University. Biomaterial surface properties including chemistry, topography, and energy regulate cell response. Rough titanium (Ti) surfaces with high surface energy modulate inflammatory cytokine release from stem cells and osteoblasts, but the effect of these modifications on immune cells, including macrophages, is less understood. Macrophages are among the first cells to interact with the biomaterial. Macrophages can activate as pro-inflammatory (M1) or anti-inflammatory (M2), which controls the inflammatory microenvironment and host response to a material. Our aim was to examine the effect of surface microstructure and energy on macrophage activation and cytokine production in vitro using primary C57Bl/6 mouse macrophages. Cells were cultured on smooth Ti, rough hydrophobic and hydrophilic Ti surfaces with tissue culture polystyrene (TCPS) control. Pro-inflammatory IL-1β, IL-6, and TNFα were lower on rough hydrophilic Ti surfaces in comparison to TCPS and hydrophobic Ti surfaces, Rough hydrophilic surfaces induced an M2 phenotype characterized by IL-4, IL-10, and IL-13 secretion. Release of pro-inflammatory and anti-inflammatory cytokines was similar between smooth and rough hydrophobic surfaces. Here we demonstrate that macrophage polarization can be controlled through surface modifications, specifically roughness and energy, to promote anti-inflammatory M2 polarization.

DEPENDENCE OF SURFACE NANOSTRUCTURES ON HYDROPHILICITY FOR ENHANCED OSTEOBLASTIC DIFFERENTIATION AND MATURATION ON TITANIUM IMPLANTS. Ethan M. Lotza<sup>1</sup>, Rene Olivares-Navarretea<sup>1</sup>, Sharon Hyzya<sup>1</sup>, Zvi Schwartza,<sup>2</sup> & Barbara D. Boyana,<sup>3</sup> Department of Biomedical Engineering, Virginia Commonwealth University, Richmond VA, <sup>2</sup>Department of Periodontics, University of Texas Health Science Center at San Antonio, San Antonio, TX, <sup>3</sup>Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA. Control of the microenvironment surrounding an implant to facilitate repair and

function of bone tissue can be achieved by modifying the implant surface. Studies have highlighted the importance of nanostructured and hydrophilic implant surfaces to enhance bone repair. Although it has been shown that surface roughness has an effect on hydrophilicity, no attempt has been made to distinguish between their effects on the process of healing and osseointegration. To test this, mesenchymal stem cells (MSCs) and normal human osteoblasts (OBs) were cultured separately on either tissue culture polystyrene (TCPS), non-nanostructured/hydrophobic SLA (grit-blasted/acid-etched treatment), nanostructured/hydrophilic modSLA (SLA with 0.9% saline storage), or nanostructured/hydrophobic SLAnano (aged modSLA, removed from saline, and stored dry). Compared to TCPS, osteoblastic differentiation and maturation were enhanced in both MSCs and OBs while inflammatory markers decreased. Among surfaces, modSLA had the highest levels of osteoblastic markers while few differences were detected between SLA and SLAnano. These findings suggest that the enhancement of osseointegration by nanostructures is dependent on the surface hydrophilicity of the titanium implant.

### Posters

EFFECTS OF GLUCOSE CONCENTRATION ON FIBRILLOGENESIS IN BREAST EPITHELIAL CELLS. B. A. Martinez<sup>1</sup>, L. Griggs<sup>1</sup>, M. Zhao<sup>2</sup>, L. Elmore<sup>2</sup>, & C. A. Lemmon', Department of Biomedical Engineering, School of Engineering and <sup>2</sup>Department of Pathology, Virginia Commonwealth University. Fibronectin (FN) is a soluble glycoprotein that makes up a major component of the extracellular matrix in tissues. Studies have shown its importance in wound healing and embryonic development. FN expression is low in most adult healthy tissues; however, in many cancers FN expression is dramatically increased. Studies from rat mesangial kidney cells have shown a linkage between high glucose concentration and increased FN expression. A common hallmark of many cancers is increased glucose transport into the cell as well as increased glucose metabolism within the cell. We thus hypothesize that increased glucose metabolism may be a driving factor in increased FN assembly in tumors. To investigate this, healthy mammary epithelial cells, premalignant epithelial cells, and malignant epithelial cells were cultured using various glucose concentrations. Previous studies have shown that assembly of FN drives a process known as Epithelial to Mesenchymal Transition (EMT), which has been implicated in cancer. Results show that glucose appears to have a biphasic effect on healthy epithelial cells: at low concentrations, epithelial cell-cell junctions start to break down, but cells don't appear mesenchymal. At higher concentrations, cells start to appear more mesenchymal. In contrast, pre-malignant cells show an opposite relationship: low glucose induced a more mesenchymal phenotype. These results suggest a complex relationship between cell malignancy, extracellular glucose, FN assembly, and EMT. This research was funded by the IMSD program at VCU CoHD.

CHANGING DIFFERENTIATION PATHWAYS OF HUMAN MESENCHYMAL STEM CELLS BY ALTERING FIBRONECTIN ASSEMBLY. T. J. Petet, J. Narang & C. A. Lemmon, Dept of BME, VCU. Stem cells differentiate when exposed to relevant growth factors and chemical signals, and also differentiate in response to the stiffness of their substrate. However, the link between mechanical and chemical signals

is not well understood. The aim of this study is to investigate whether assembly of the extracellular matrix protein fibronectin (FN) serves as an integration point for mechanical and chemical signals, and that differentiation depends on the assembly of FN. It is hypothesized that by blocking FN assembly, substrate stiffness can be high yet the stem cells will undergo differentiation as though they were on a soft surface. In order to test this, we cultured human mesenchymal stem cells on stiff (glass) FN-coated substrates in the presence of a 1:1 mix of adipogenic and osteogenic differentiation factors. FN was inhibited with a fragment of the bacterial protein F1 adhesin (FUD), which has previously been shown to inhibit assembly of FN into fibrils that are a key component of the extracellular matrix. Cells were cultured for 10 days to allow the cells to differentiate and then were fixed and stained for differentiation markers. Preliminary data supports the hypothesis: FUD blocked assembly of FN and drove differentiation towards an adipocyte lineage, while untreated samples showed an osteogenic lineage. Future experiments will use surfaces with varying stiffness to determine whether the hypothesis holds across the range of substrate mechanical properties.

COMMON CORE AHP APP. David S. Parker, Biomedical Engineering, Virginia Commonwealth University. In the School of Allied Health at VCU assessing the students as they progress through practical training is a very important tool. It identifies areas for the student where he or she needs to improve as well as areas in which that student is doing well. Currently both the students as well as the teacher are doing the evaluations on paper. The problem caused by this is that evaluations filled out can easily be lost, or not stored properly. As a result, the progress of a student over time cannot be tracked very well. To fix this issue an application is being created to conduct as well as store the evaluations by the student as well as the teacher. This app uses the set of questions that was created by Dr. Dianne Simmons to assess the student's progress. The results of these questions will then be stored in a web database using Wi-Fi, so that the student can easily compare current results with previous results. Also, the student will be provided an area to tell the teacher of areas they thought they did well in, and areas they need to improve in. Another thing that this application will allow is for the student and the teacher to share their evaluations with each other. This will be achieved by using Bluetooth to share the information. Because of this the student will be allowed to compare their own assessment to the teachers' assessment. This can open up a discussion between the student and the teacher on the things that they need to improve as well as things they need to keep doing well.

MODELING WATER FLOW THROUGH GRAVEL NESTS USING ELECTRICAL CIRCUIT ANALYSIS. N. W. Brigle<sup>1</sup> & E. G. Maurakis<sup>2,3</sup>, <sup>1</sup>Dept. Biomedical Engineering, Virginia Commonwealth University, <sup>2</sup>Biology Dept., University of Richmond & <sup>3</sup>Science Museum of Virginia. Using electrical circuit analysis (ECA), resistance and water current velocity of anterior, middle, and posterior sections of nests of *Nocomis micropogon* and *Nocomis leptochephalus* were determined. Each section's resistance was plotted against its corresponding cross-sectional area. Analysis of these plots yielded two equations (Universal Water Equation (UWE) and Universal Nest Equation (UNE)) for water current velocity. For *N. micropogon* and N. *leptocephalus*, the UNE yielded the best results for determining water current velocity around anterior (0% and 0.02% error respectively) and middle (0% and 0% error respectively) sections

of nests, while UWE yielded best results for determining water velocity for posterior (0.02% and 0.54% respectively) section of nests. Compared to water current velocity calculated by ECA, UNE yielded best results for determining water current velocity through anterior (0.01% and 0.18% error, respectively) and middle (0.12% and 0.02% error, respectively) sections of nests, while UWE yielded best results for determining water velocity for posterior (0.04% and 0.02% respectively) sections of nests. UWE and UNE can be applied to nests of other species of *Nocomis* and those of *Semotilus* and *Exoglossum* after nest micro-water currents field evaluation.

ROBOTIC PLATFORM TO GUIDE AND ASSIST INFANTS, POSSESSING VISUAL IMPAIRMENTS, WITH CRAWLING AND EXPLORATION. Muhammed M. Naqvi, Sean W. Megahan, David S. Parker, Dr. Dianne T.V. Pawluk, Ross A. Petrella & Dr. Peter Pidcoc, Department of Biomedical Engineering, Virginia Commonwealth University, Richmond VA 23284-2006. Without visual stimulus, infants who are blind or visually impaired (BVI) lack an opportunity to develop their motor, cognitive, and social skills. To solve this problem a robotic platform was created teach and foster independent exploration in BVI infants using haptic feedback and variable motor assistance. The robot platform was controlled and tracked by a smart phone application. A microcontroller on the robot platform interpreted the application data and supplied the infant vibrotactile feedback which described the distance and orientation of the target. Training paradigms were created to teach the infant to use the vibrotactile feedback and then gradually transition the infant off motor assistance. Preliminary testing showed successful functionality of the initial prototype. The robot platform and Android application designed were capable of determining distance and orientation relative to one another as well as providing haptic feedback and motor assistance to the infant. With mild improvements to the chassis, user interface and motors, the prototype would be ready for clinical trials.

#### Botany

TREE SURVIVAL IN CREATED PIEDMONT WETLANDS AND THE EFFECT OF SOIL CONDITIONS. M. Seidel & R. B. Atkinson, Dept. of Organismal and Environmental Biol., Christopher Newport Univ., Newport News VA 23606. Forested wetlands are the most frequently lost wetland type in the Eastern United States, and tree establishment efforts in created wetlands may be inadequate where survival rates are low. The purpose of this study was to evaluate survival rates using environmental variables as predictors of survival for seven woody plant species and three stocktypes. Trees were planted in March of 2009 in three created wetlands in Loudoun County, VA and survival was assessed annually during early August from 2009 to 2013. Overall survival after five years was 44.4% and survival was lowest between the first and second growing seasons. Survival of *Quercus palustris* in 1-gallon pots stocktype was highest (76.3%) and *Q. phellos* in tubeling stocktype was numerically lowest overall (7.95%). Survival rate was negatively related to organic matter content in the soil; however, depth and/or duration of hydroperiod may have influenced organic matter content. Support from the Peterson Foundation is gratefully acknowledged.

LEWIS GINTER BOTANICAL GARDEN – OUR PASSION IS CONNECTING PLANTS AND PEOPLE. Shane W. Tippett, Lewis Ginter Botanical Garden, 1800 Lakeside Avenue, Richmond VA 23228. Botanical gardens as cultural institutions and community anchors have developed in multiple countries through hundreds of years for reasons as varied as the gardens themselves. Regardless of their founding principles, many botanical gardens are now good places for the discussion of challenges which might be addressed at least in part by plant-based solutions. What are challenges with plant-based solutions? Healthy air to breathe; sustainable, nutritious food to eat; limited water resources to steward; effects of climate change to mitigate; a changing world to understand and tend. Why a botanical garden, our garden? Lewis Ginter frequently is viewed as practical and educational on divisive issues, we draw a third of a million guests (students) annually, and the guest experience is engaging, interactive and memorable.

VEGETATION PATTERNS AND HYDROLOGIC REGIMES IN THE GREAT DISMAL SWAMP. Kristina M. Kowalski & Robert B. Atkinson, Dept. of Organismal and Environmental Biology, Christopher Newport Univ., Newport News VA 23606. Draining activities in the Great Dismal Swamp (GDS) began with small ditches in the colonial era, however during the industrial era the ditches were extensively deepened, which dramatically altered the hydrology of the swamp. Since 1974, the GDS National Wildlife Refuge has ceased ditching and began managing the swamp for restoration of hydrology and natural plant communities. In 2013, two new weirs were installed on the eastern side of the GDS, which influences the hydrology of the proximate management units. The purpose of this study was to [1] assess the vegetative communities within management blocks soon to be influenced by hydrologic restoration [2] compare water table depth to vegetative community structure. Eleven 10-m<sup>2</sup> plots were established adjacent to well locations. Tree, shrub, herb, and vine strata were surveyed individually using estimated percent aerial cover. Percent cover and wetland indicator score were used to calculate weighted averages. Mean depth to the water table (MDWT) was calculated for the period of June 6 to July 21, 2013. Grand mean weighted averages among strata and MDWT were positively related ( $R^2 = 0.34$ ). Weighted averages among tree, shrub, herb and vine strata varied in relation to MDWT ( $R^2 = 0.45, 0.14$ , 0.10, and 0.27 respectively). These results suggest that water levels significantly influence the plant community; however the relationship is not consistent among strata and responses to increased water levels may vary.

FLORAL ANATOMY AND COROLLA NECTAR TUBES IN *PHYSALIS* (SOLANACEAE). <u>Deanna Dong</u> & W. John Hayden, Dept. of Biol., Univ. of Richmond, Richmond VA 23173. We report on floral anatomy of five *Physalis* species from eastern North America. Tissues were prepared for light and scanning electron microscopy using standard techniques. All species studied possess flowers with five fused sepals that become accrescent in fruit, five fused yellow petals often with dense pubescence within the throat and dark spots nearby, and five epipetalous stamens. Flowers have a ring-like nectary below a two- or three-carpellate superior ovary. Anthers include sterile placentoids and stomium cells bearing crystal sand. Axile placentas bear numerous unitegmic anatropous ovules. Floral organs initiate in acropetal order as separate elements before fusing (connation). Notably, petal to stamen

adnation initiates (before petal-petal connation) as contact below tips of stamen primordia and proceeds basipetally; separate stamen primordium tips elongate, forming anthers. Corollas of all species studied possess a feature previously not well documented, capillary nectar "tubes;" these are anatomical grooves adaxial to petal midveins that extend from petal bases (adjacent to the nectary) to points approximately midway on the corolla limb. Nectar tubes develop as gaps between laterally adjacent mesophyll cells that divide and thicken petal gases, comparable to adaxial meristems of leaf ontogeny. Nectar tubes end at dark spots on the corolla, inferred to serve as nectar guides for pollinators, *Capsicum* and *Jaltomata* are reported to have similar nectar tubes.

ANATOMY ANDMORPHOLOGY ΟF THE SUBTERRANEAN BENGHALENSIS CLEISTOGAMOUS FLOWERS OF COMMELINA (COMMELINACEAE). Elizabeth C. Fagan & W. John Hayden, Dept. of Biol., Univ. of Richmond, Richmond, VA 23173. Anatomical studies of the federally listed noxious weed Commelina benghalensis were undertaken to document structural and developmental details of its subterranean cleistogamous flowers. Specimens were collected on the University of Richmond campus in October 2012 and prepared for study with light and scanning electron microscopy using standard techniques. Results confirm and extend earlier literature. Cleistogamous spathes are solitary, axillary, subtended by a leafy scale, and bear a single bisexual flower. Three sepals enclose other floral organs, including three petals, three posterior stamens that are nonfunctional staminodes, three anterior stamens that are functional, and a three-carpellate gynoccium. Functional stamens have a well-developed endothecium and pollen grains are enveloped in an amoeboid tapetum. Ovules are orthotropic, bitegmic, and crassinucellate. Details of pollination within these closed flowers remains obscure; an earlier report noted pollen grains germinating on stigmas, an observation we cannot confirm. In some flowers, anthers were found in direct contact with stigmas, suggesting the possibility that pollen could germinate while still inside the anthers of cleistogamous flowers. Cleistogamous flowers produce dimorphic seeds, one large seed in the posterior locule and two small seeds in the anterior locules.

ADDITION OF BRYOPHYTE RECORDS TO THE DIGITAL ATLAS OF THE VIRGINIA FLORA AND OTHER CRYPTOGAMIC CURATORIAL NEWS FROM THE UNIVERSITY OF RICHMOND HERBARIUM. Catherine Sinclair, Michelle Ferrell & W. John Hayden, Dept. of Biol., Univ. of Richmond, Richmond, VA 23173. David A. Breil of Longwood University had summarized his knowledge of Virginia bryophytes in the form of a hardcopy atlas that remained unpublished upon his death in 1997. Breil's Bryophytes of Virginia Atlas contained records of occurrence by county for 148 species of liverworts and hornworts classified in 36 families and 349 species of moss classified in 45 families. These records are now incorporated into the Digital Atlas of the Virginia Flora website maintained by the Virginia Botanical Associates. To the extent possible, we have endeavored to update Breil's data using currently accepted nomenclature and current concepts of family placement. Other curatorial activity involving cryptogamic collections at the University of Richmond Herbarium (URV) has focused on lichens, notably: 1) integration of previously separate collections made by E. S. Luttrell in the early 1940's, (1202 specimens), K. L. Poff in

the early 1960's (210 specimens) and W. J. Hayden in recent decades (140 specimens); 2) identification of numerous "indets;" 3) replacing aged packets; 4) addition of 257 specimens collected by M. Ferrell; and 5) application of bar codes to each specimen. URV bryophyte and lichen collections have been loaned to and returned from DUKE Univ. as part of an NSF-funded herbarium digital imaging effort.

THE FLORA OF VIRGINIA PROJECT: A 2013-2014 PROGRESS UPDATE. Marion B. Lobstein, Northern Virginia Community College. Virginia, for its landmass, has the most diversity of vascular plant species of any state in the United States. The Colony of Virginia had the first flora, Flora Virginica, of any of the colonies which was last updated in 1762. Since 1926 the Virginia Academy of Science has supported efforts to produce a modern Flora of Virginia. In 2001 the Foundation of the Flora of Virginia, Inc, was formed in 2001to realize this goal. The Flora of Virginia was published in December 2012. The second printing with corrections was published December 2013. The Foundation of the Flora of Virginia project will continue to function to accomplish future goal of the Project. One of those goals is to development of teaching and learning modules. Marion Lobstein shared details of her efforts to develop workshops and materials to facilitate the use of the new Flora of Virginia by interested adult groups.

THE RELATIONSHIP BETWEEN RIPARIAN ZONE WIDTH AND FLORISTIC QUALITY ALONG STREAMS IN SHENANDOAH COUNTY, VIRGINIA. Jamie D. Smith & Conley K. McMullen, Dept. of Biology, James Madison University, Harrisonburg VA 22807. Riparian zones harbor an above average plant biodiversity. This biodiversity is increasingly threatened by invasive species and human disturbance such as deforestation from agriculture and urban development. This study will determine the relationship between the size of the riparian zone and the floristic quality of its vegetation. A bioassessment tool, which utilizes plant community characteristics will be used to obtain an estimate of habitat quality. This estimate, in turn will determine whether larger riparian zones provide more protection from the impacts of disturbance and provide higher quality habitats for plants. Preliminary data from vegetation plots show that there is a trend towards higher floristic quality in larger riparian areas. However, the number of non-native species in a particular area appears to be unchanged as riparian zone size increases, indicating that although larger riparian areas do harbor more native and conservative species, they are not effective at limiting invasion by non-native species.

FLORISTIC SURVEY OF THE SMITH CREEK RESTORATION AREA, ROCKINGHAM COUNTY, VIRGINIA. Allison N. Welborn & Conley K. McMullen, Dept. of Biol., James Madison Univ., Harrisonburg, VA 22807. Smith Creek Restoration Area, in Rockingham County, VA, comprises a 1.5-mi section of creek and a 66-ft buffer zone along either side. Prior to the beginning of this study, for ca. 200 years, the property had been used for agriculture/pasture. As a result, the creek had become unsuitable as a habitat for native brook trout, and most of the native plants in the immediate area were lost. In 2006, the land bordering the creek was made available for a restoration project that planted thousands of trees in the hopes of eventually returning the area to its natural condition. The purpose of the research described here

is to catalog the native vascular plant species that occur in this area, thus providing baseline data for future studies that might occur as the landscape changes due to the tree plantings and the absence of agricultural and grazing pressures. The 254 species found in the area so far represent 158 genera and 52 families, with ca. 58% being native to Virginia and ca. 41% non-native.

FIRST REPORT OF LEAVENWORTHIA (BRASSICACEAE) IN VIRGINIA. Conley K. McMullen & Brinton E. Domangue, Dept. of Biology, James Madison University, Harrisonburg VA 22807. During a survey of the vascular flora of Shenandoah County, VA, a population of Leavenworthia uniflora (Michaux) Britton (Michaux' gladecress) was discovered on 22 March 2012. This collection represents the first report of Leavenworthia in Virginia. Leavenworthia comprises eight native species, primarily located in the southeastern United States, Leavenworthia uniflora has previously been reported from Alabama, Arkansas, Georgia, Indiana, Kentucky, Missouri, Ohio, and Tennessee. Consequently, the population reported here makes Virginia the easternmost state in which Leavenworthia is found. Leavenworthia uniflora typically inhabits rocky ledges, cedar glades, pastures, roadsides, old fields, thin soil on limestone beds, and seeps on limestone rubble. Fittingly, the described population is found adjacent to a limestone quarry. This population of perhaps 50 individuals will be monitored to determine its persistence and possible spread.

COMPARISON OF ANTIOXIDANTS FROM COMMERCIAL FRESH AND DRIED HERBS. Jessica C. Wagner, Michael H. Renfroe, Harley J. Burton & Michele L. Barber, Dept. of Biol., James Madison Univ., Harrisonburg VA 22807. Commercially purchased herbs and spices were analyzed for antioxidant content. Antioxidants are an important component of the human diet and previous research has demonstrated that antioxidants help reduce incidence of cancer, atherosclerosis, scrility, and other diseases associated with aging. We analyzed hydrophilic antioxidant content in fresh tissues from cilantro, mint and parsley. We analyzed hydrophilic antioxidant content in dried tissues from basil, Mediterranean basil, parsley, cilantro, rosemary, gourmet rosemary, rubbed sage, oregano, chives, and thyme. Among fresh herbs, significant differences were found from plant to plant within species as well as between species. Of the observed herbs, mint had the greatest concentration of hydrophilic antioxidants. Among dried herbs and spices, rosemary contained the greatest concentration of hydrophilic antioxidant, followed by basil, thyme, gourmet rosemary, and sage. Mediterranean basil and oregano contained significantly less antioxidant concentration. Cilantro, parsley, and chives contained the least concentrations of hydrophilic antioxidants. Lipophilic antioxidant concentrations were not examined in this study. These results may prove useful to dieticians and the general public when choosing a diet rich in antioxidants.

AN UPDATED PHYLOGENY OF THE TRIBE CHIOCOCCEAE (RUBIACEAE)-NEW INSIGHTS INTO TAXONOMIC RELATIONSHIPS. S. K. Paudyal<sup>1</sup>, P. G. Delprete<sup>2</sup> & T. J. Motley (posthumous)<sup>1</sup>, <sup>1</sup>Old Dominion University, Norfolk, VA 23529 and <sup>2</sup>Herbier de Guyane, Cayenne Cedex, French Guiana, France. The Chiococceae is a monophyletic tribe in the subfamily Cinchonoideae (Rubiaceae) that includes 29 genera and over 200 species primarily distributed in the Neotropics but also

occurring in the islands of western Pacific Ocean with no members occurring on the expansive Pacific plate, thus exhibiting an intriguing biogeographic disjunction. Although monophyly of the tribe has been established by recent molecular phylogenies, many inter-generic and infra-generic relationships remain poorly resolved and therefore questions on the taxonomic affinities and evolutionary relationships within the group are yet to be fully understood. A number of larger genera have been shown to be paraor polyphyletic as currently treated. To resolve these taxonomic questions, we significantly expanded taxa sampling and used additional DNA sequence data. Based on the analyses of molecular sequence data of two nuclear (ETS, ITS) and two chloroplast (petD, trnL-F) loci obtained from 132 species representing 27 genera, here we present a comprehensive phylogeny of the tribe Chiococceae. Our results provide new insights into evolutionary relationships, thus suggesting need for a comprehensive revision of certain infra-generic classifications within Chiococceae.

# Posters

WHITE CEDAR (THUJA OCCIDENTALIS) SAPLINGS IN THE NORTHERN SHENANDOAH VALLEY, VIRGINIA. Kirby Talbert & Joshua Kincaid, Environmental Studies Program, Shenandoah University, Winchester, VA 22601. White cedar (Thuja occidentalis) is a long-lived and shade tolerant coniferous tree species of eastern North America. The geographic range of white cedar extends from southeastern Canada to the New England and Great Lakes regions. Scattered, disjunct populations exist southward into North Carolina and Tennessee. While white cedar grows on a variety of organic and mineral soils, it tends to grow best on limestonederived soils. In the Shenandoah Valley, white cedar forests are typically found on steep, north or west-facing slopes with underlying limestone or dolomite bedrock. According to the VA Department of Conservation and Recreation, white cedar populations are found in mixed forest stands, and are extremely rare in Virginia. Because white cedar forests are rare in the Shenandoah Valley region, more research is necessary to understand the composition, structure, and regeneration dynamics of these forests. A total of 10 white cedar saplings were destructively sampled to examine structural and growth characteristics. Preliminary results suggest that white cedar saplings growing in sunny conditions are larger and taller than those growing in the shade. A mean difference of 23 years is found between the 0 m and 1.4 m sampling heights. White cedar sapling growth is also correlated with current and previous year temperature and precipitation.

INDUCING GERMINATION/PROPAGATION IN MOUNTAIN LAUREL, KALMIA LATIFOLIA. L. J. Queitzsch & Stephen W. Fuller, Dept. of Biol., Univ. of Mary Washington, Fredericksburg, VA 22401. Mountain Laurel, Kalmia latifolia, is a flowering shrub native to the Eastern United States. It thrives in acidic soil and shade, although during germination and propagation Kalmia latifolia enjoys constant sunlight. Mountain laurel is a popular landscaping plant but is difficult to cultivate in greenhouse and laboratory environments. This experiment attempts to propagate seeds and induce rooting in cuttings of Kalmia latifolia. Seeds of Kalmia latifolia were successfully propagated in soil mixtures [1] containing 4 parts peat moss and 1 part perlite/vermiculite, [2] containing 1 part potting soil, 1 part sand, 1 part peat moss, and 1 part

perlite/vermiculite, [3] containing 1 part potting soil and 1 part peat moss, and [4] containing 1 part sand and 1 part peat moss. Seeds seem to be most successful in soil [4]. Stem cuttings of Mountain Laurel plants failed to root and propagate in all soils except for soil [2] containing 1 part potting soil, 1 part sand, 1 part peat moss, and 1 part perlite/vermiculite. This would indicate that the best possible soil mixture for propagating *Kalmia latifolia* changes depending on whether the plant is being propagated from seeds or stem cuttings. Of the hundreds of seeds sown, only 94 achieved a size where they could be transplanted, and of those, 70 achieved a size of 3-4 cm. After a year of care in a greenhouse, only 15 have survived. Once more establishing the difficulty of propagating this popular horticultural plant.

DEVELOPING AND EVALUATING NOVEL NUCLEAR MARKERS FOR PHYLOGENETIC RECONSTRUCTION IN THE MYRRH GENUS, COMMIPHORA JACQ. (BURSERACEAE). Kiera A. Coy, Andrea Weeks & Morgan Gostel, Dept. of Environmental Science and Policy, George Mason Univ., Fairfax VA. 22030. To date, widely available nuclear genetic markers (e.g., ETS, ITS) have not provided sufficient variation to fully resolve the evolutionary relationships among the ca. 200 species of Commiphora. Here we use genomic resources to expand the range of genetic markers that can be used for comparative phylogenetic analyses. This poster describes the development and evaluation of these markers from a target pool of about 950 putative shared single-copy nuclear genes using Madagascan species of Commiphora to test them. Development of these markers followed a bioinformatics process that compares the transcriptome of Bursera simaruha against a pool of putative single-copy, orthologous nuclear genes shared across angiosperms. Our analysis identified a subset of approximately 240 genes that aligned to the B. simaruba and our target gene pool. We demonstrate the effectiveness of a subset of markers for comparative phylogenetic analyses and propose criteria for future research that will consist of primer optimization, size standardization of target products, and sequencing the targets using microfluidic PCR and multiplexed next-generation sequencing. These gene regions may be useful for phylogenetic analysis in allowing us to better understand the rapid diversification of Commiphora in Madagascar and resolving challenging species-level relationships in other angiosperm groups beyond Commiphora and Burseraceae. We will develop test these markers more broadly in other angiosperm lineages for comparative phylogenetics at broad and shallow-scales.

PROGRESS IN PHYLOGENETIC STUDY OF NORTH **AMERICAN** MELAMPYRUM LINEARE (OROBANCHACEAE). Karoline Oldham, School of Systems Biol., George Mason Univ., Fairfax, VA 22030 & Andrea Weeks, Environmental Science and Policy, George Mason Univ., Fairfax VA 22030. Melampyrum lineare (Orobanchaceae) is the sole North American species of its genus, which comprises another 34 species in Eurasia. Previous literature proposes two competing historical biogeographical hypotheses: 1) M. lineare shares its most recent common ancestor with a European species, M. pratense; 2) M. lineare shares its most recent common ancestor with the Asian species M. arcuatum, or M. laxum var. arcuatum. However, these hypotheses were based solely on morphological observations and do not consider DNA sequence data. Comparison of ITS sequences from M. lineare and other members of the Rhinantheae tribe suggests that M. lineare

is not sister to *M. pratense*. Rather, this analysis provides mixed support for the competing hypotheses of European versus Asian ancestry.

# Chemistry

CHEMICAL PROBES FOR PROTEIN N-TERMINAL METHYLTRANSFERASE. Rong Huang, Gang Zhang, Yunfei Mao, & Stacic Lynn Richardson, Department of Medicinal Chemistry, Institute for Structural Biology and Drug Discovery, Virginia Commonwealth University, Richmond VA 23219. The objective of this study is to develop chemical probes for N-terminal RCC1 methyltransferase (NRMT1), NRMT transfers methyl groups from S-adenosyl methionine to the N-terminal alpha-amine of proteins. It plays an important role in regulating protein-DNA binding and proteinprotein interactions, However, there is no chemical tool available to further characterize NRMT biological and pharmacological roles. We have characterized the kinetic mechanism of NRMT1 using recombinant NRMT1 via a MALDI-MS method. Our results indicated that NRMT1 catalysis underwent a formation a ternary complex. Based on this mechanism, we adopted a bisubstrate strategy to design NRMT inhibitors to covalently link an N-adenosyl methionine (NAM) to a peptide derived from Nterminal protein substrate. We have successfully synthesized a series of bisubstrate analogues and determined their inhibitory activities via a fluorescent competitive assay. Among them, SPKR-NAM exhibits a highest inhibitory activity with an IC<sub>50</sub> of 13.4 uM. As negative controls, both NAM-triazole and triazole-peptide did not show any significant inhibition at 100 uM. In conclusion, our bisubstrate inhibitors showed more than 10-fold inhibitory activities and they can be used as chemical tools for future study of NRMT1.

THE BLACK POLYMORPH OF TTF-CA: TTF POLYMORPHISM AND SOLVENT EFFECTS IN MECHANOCHEMICAL AND VAPOR DIGESTION SYNTHESES. FT-IR, CRYSTAL PACKING AND ELECTRONIC STRUCTURE. Silvina Pagola', Saul H. Lapidus<sup>2</sup>, & Amit Naik<sup>3</sup>, Department of Applied Science, College of William and Mary, Williamsburg, VA, 23187, Dept. of Physics & Astronomy, Stony Brook University, Stony Brook, NY, 11794, & Thomas Nelson Community College, Hampton, VA, 23666. Tetrathiafulvalene-chloranil (TTF-CA) was synthesized by liquid assisted grinding (LAG) and vapor digestion (VD), largely reducing the use of reaction solvents. The effects of the small quantities of LAG and VD solvents towards the formation of a particular TTF-CA polymorph were studied from the orange and brown tetrathiafulvalene (TTF) polymorphs as reactants. A high solvent polarity favors the formation of the ionic (black) TTF-CA vs. the quasi-neutral green form, whereas the crystal structure and crystal habit of the orange TTF also favors the formation of the black TTF-CA. The crystal structure of the black TTF-CA was determined from synchrotron X-ray powder diffraction and it consists of dimerized TTF+• and CA-• radical ions, in agreement with room temperature magnetic susceptibility measurements indicating the material is diamagnetic. The black TTF-CA does not undergo a phase transition in the range 298K-20K. FT-IR showed that the compound is a semiconductor with a band gap of ~0.198 eV and it remains ionic at low temperatures. Band structure calculations are in good agreement with the measured band gap.

TESTING FOR INTERFERING SUBSTANCES USING OIML RECOMMENDED CONCENTRATIONS ON VIRGINIA INTOX EC/IR II BREATH TEST INSTRUMENTS. T. L. Neece, Jr., M. S. Kennedy & A. B. Lohmann, Virginia Department of Forensic Science, Richmond VA 23141. The International Organization for Legal Metrology (OIML) has established recommendations for breath alcohol testing related to the interference of chemicals that may occur in human breath at the time of sampling. Acctone, methanol, and isopropanol are currently listed as possible interferents and were tested on the Intox EC/IR II by the Virginia Department of Forensic Science, Acetaldehyde was also tested in this study as it was previously listed by OIML and examined by the laboratory. These chemicals were tested at, above, and below the levels recommended by OIML. Results of this testing demonstrate the Intox EC/IR II with Virginia firmware meets the OIML interfering substance recommendations to ensure accurate and precise testing of evidential breath samples in the Commonwealth of Virginia.

EVALUATION OF THE EFFECT OF INVISAILGN ON MOUTH ALCOHOL RETENTION ON THE INTOX EC/IR II BREATH TESTING INSTRUMENT. M. M. Billeter, M. S. Kennedy & A. B. Lohmann, Virginia Department of Forensic Science, Richmond, VA 23219. The presence of an Invisalign device was evaluated to determine if it will retain mouth alcohol and interfere with the accuracy of a breath test. The rate of mouth alcohol elimination was observed in one subject wearing Invisalign. The subject was tested under two conditions. The first test was while wearing the device. The subject rinsed her mouth with a 43% ethanol solution and was tested immediately on the Intox EC/IR II Breath Instrument. In the second test, the subject rinsed with the alcohol beverage and then placed the Invisalign in her mouth and was again tested immediately. It was observed that after 10 minutes, no residual mouth alcohol remained. This confirmed that the 20 minute observation period prior to performing a breath test ensures that there is no effect from residual mouth alcohol when wearing an Invisalign device.

THE EFFECT OF STORM WATER RETENTION PONDS ON WATER QUALITY IN AN URBANIZED AREA AS INDICATED BY ANION CONCENTRATIONS. C. Aneroussos & M. Howard, Department of Chemistry, Virginia Wesleyan College, Norfolk, VA 23502. The focus of the current study is to establish a baseline of sampling and analysis methods for future research while simultaneously collecting and analyzing preliminary data for the long term goal of increasing storm water pond efficiency through the use of vegetation. Storm water retention ponds act as collection points (or buffer areas) for run-off water, before the water travels to natural water sources such as streams, lakes or oceans. Past research suggests that storm water retentions ponds affect anion concentrations, and therefore water quality in an urbanized area. The anion concentrations present in the runoff water of urbanized areas is largely influenced by human activity. Major anions such as chloride, phosphate and sulfate come from a number of sources including farming activity, pollution emission, natural ecological activities such as excretion and many more. Significant imbalances in the anion concentration can cause a plethora of negative from harmful algal blooms, to dead zones, to ecosystem (resource) distortion. The research being discussed took place over the course of approximately 4 weeks. Water samples were collected from

storm water retention ponds located on the campus of Virginia Wesleyan College and were analyzed by ion chromatography to determine anion concentrations. The research was conducted during the winter season in order to limit the effect of biological activity (which is severely reduced in the winter months) and explores sampling methodology, methods of analysis and establishes a baseline for reference as the project continues.

AN INVESTIGATION OF THE COMPOSITION OF GLAZE AND CERAMIC PIECES FROM NICARAGUA. A. Ibarra & M. Howard, Department of Chemistry, Virginia Wesleyan College, Norfolk, VA 23502. The purpose of this experiment is to examine the compositional variation in glazed and unglazed ceramic samples from collection sites in Nicaragua. Physical and chemical properties are eompared using seanning electron microscopy and energy dispersive spectroscopy (SEM-EDS). Trace elements are also analyzed using flame atomic absorption spectrometry (FAAS). Glazes and eeramics used in eommercial production of pottery versus traditionally produced vessels and those sold by local Nicaraguan street vendors are compared to identify similarities and differences in composition.

PROGRESS TOWARDS THE SYNTHESIS OF Α SERIES OF TPA CENTROSYMMETRIC VINYLENE-BRIDGED ARYL STYRYL HETEROCYCLIC D-PI-D SYSTEMS. Michelle K. Waddell, Department of Chemistry, Hampton University, Hampton, VA 23668. As a result of their unique delocalized  $\pi$ -electron systems and fascinating optical and electro-optical properties, conjugated organic molecules and polymers are making significant contributions to the emerging technologies of molecular electronics and photonics. One area of research interests is the design, synthesis, and investigation of conjugated organic compounds with new molecular architectures to be used as electronic and photonic materials multichromophoric benzenes. Organic molecular systems with large multi-photon absorption, MPA, in particular, Two Photon Absorbance (TPA) eross-sections, have a broad spectrum of technological applications. The use of organic methods for the synthesis of these compounds is ideal; it permits not only the tailoring of molecular structures to enhance specific properties, but also the tuning of physical characteristics via changes in functionality. Progress towards the synthesis of 2,5-bis(4'-N,Ndicthylaminostyryl)thiophene (BEST), 2,5-bis(4'-N,N-diethylaminostyryl)furan (BESF) and 2,5-bis(4'-N,N-diethylaminostyryl) pyrrole (BESP) was performed with characterization of these compounds. These compounds were synthesized in a three step process which readily allows for the interchange of reagents to generate a library of compounds. Fluorescence absorption spectra of these compounds will be compared to computational models to validate the results of theoretical studies.

INTERACTION OF METAL NANOPARTICLES WITH THIOL AND THIOETHER-CONTAINING COMPOUNDS. S. Babu¹, Jaetae Seo² & M. O. Claville¹, ¹Department of Chemistry and ²Department of Physics, School of Science, Hampton University, Hampton, VA 23668. Gold nanoparticles (Au NPs) have vast applications in drug therapy because of their unique optical, electronic and molecular properties. We present preliminary findings regarding the interaction of Au NPs with methionyl compounds, L-Methionine(Met), D/L-Met, N-Acetyl-L-Met, L-Met Ethyl Ester, and Met-Glycine. The average sizes of the eight Au NPs used in the study are ~5, ~10, ~15, ~20, ~30,

~32, ~35, and ~40 nm diameters. The UV-Vis spectral studies showed that Au NPs exhibit a strong plasmon at ~530 nm while the methionyl compounds displayed an additional plasmon band at ~785 nm, indicating the formation of Au NPs aggregates. Methionine was found to aggregate Au NPs (conc. 3.487E<sup>-10</sup> M) with size ~35 nm at higher concentration (final 0.125 M) with the color change from red to blue. While lower concentration of L-Met and D/L-Met did not aggregate immediately, N-terminal (N-Acetyl-L-Met) and C-terminal protected Met (Met Ethyl Ester) readily formed Au NPs aggregates. Methionine-Glycine dipeptide was only slightly better than D/L-Met at inducing aggregation of Au NPs. The results provide insight into the impact of NP size, peptide sequence, and concentration of NPs, on aggregation. We strongly believe aggregation size and kinetics may have a role in the etiology of cellular response which needs to be systematically evaluated. [Supported by the National Science Foundation-Faculty Early Career Development (CAREER) Award, CHE-0847742 and ACE Implementation Award NSF HRD-1238838]

LEAVING GROUPS IN SUBSTITUTION AND ELIMINATION REACTIONS: AN UNEXPECTED JOURNEY. Charles M. Bump, Department of Chemistry, Hampton University, Hampton, VA 23668. Calculated (B3LYP – 3-21G) thermodynamics of competing  $S_N 2$  and E2 reactions are consistent with textbook descriptions of the competing reactions in concluding that elimination is thermodynamically favored at higher temperature. This is not a surprise since the entropy change for substitution reactions is small while the entropy change in eliminations is large. At higher temperatures, the T $\Delta S$  term in the definition of Gibbs free energy ( $\Delta G = \Delta H - T\Delta S$ ) is of minor importance in substitution reactions since  $\Delta S$  is nearly zero while that term becomes increasingly larger in elimination reactions. A plot of  $\Delta G$  as a function of temperature reveals a common temperature above which elimination is favored – regardless of leaving group. A steeper (more negative) slope of the  $\Delta G$  vs. T plot is indicative of a "better" leaving group. Or is it?

THE EFFECT OF THE PRESENCE OF Ag ON HOMO-LUMO VALUES OF THIOPHENE. C.S. McKinney & S.M. Black, Center for Materials Research, Norfolk State University, Norfolk, Virginia 23504. Transforming solar energy into electricity using photovoltaic devices provides clean, abundant and renewable energy for readily use. The alignment of HOMO and LUMO levels of donor and acceptor moieties in organic solar cells can enhance the efficiency of photovoltaic devices. We use Density Functional Theory to determine the effect of the presence of a silver dimer on the frontier orbitals of Thiophene. HOMO and LUMO energy levels and gap were obtained for the isolated Thiophene molecule, and in the presence of a silver dimer, Ag<sub>2</sub>, at different distances. Calculations were performed using the B3LYP functional, with the 6-31++G\*\* basis set chosen for the Thiophene, and cc\_pvdz\_pp for the silver dimer. Preliminary results show that the presence of Ag lowers the HOMO LUMO energy values, but no changes occur in the energy gap.

POLARIZABILITIES AND HYPERPOLARIZABILITIES OF VINYLENE - BRIDGED ARYL STYRYL SYSTEMS – SUBSTITUENT AND EXTENT OF CONJUGATION EFFECTS. <u>E. M. N. Ndip</u> & J. M. McCormick, Department of Chemistry, Hampton University, Hampton, VA 23668. An understanding of the

structure-property relationship of molecular systems of interest is important for the design of efficient organic semiconductors based on p-conjugated molecules for the fabrication of various optoelectronic devices. We have investigated the charge transport properties (band gaps and polarizabilities and hyperpolarizabilities) of forty (40) molecular systems based on varying the vinylene bridge lenths as well as permutations of three donor – acceptor pairs. Specifically, we have studied the effect of varying the donor / acceptor functionality on optoelectronic and charge transport properties was studied in donor-acceptor-thiophene systems with 1-10 vinylene bridges at the semi empirical level using MOPAC2012. These molecules all exhibit a reduced band gap, and excellent NLO properties.

(D) 
$$(H_3C)_2N$$
  $NO_2$  (A)

THE FOUNDATION FOR DESIGNING EFFECTIVE ANTI-CANCER PRO-DRUGS: A QUANTUM MECHANICAL STUDY OF THE RELATIONSHIP BETWEEN MOLECULAR STRUCTURE AND THE ENERGETICS OF BERGMAN CYCLIZATION. Arjun K. Jaini & Carol A. Parish, Department of Chemistry, University of Richmond, Richmond VA 23173. Enedignes cyclize via Bergman Cyclization to form para-benzyne, a diradical that destroys cancer cells by extracting hydrogen atoms from tumor cell DNA. Bergman Cyclization can be initiated with light or heat. An efficient way to trigger Bergman Cyclization solely in the presence of cancer cells has yet to be discovered. In year one of this study, it was concluded that tautomers were possible triggers for activating Bergman Cyclization solely in the presence of cancer cells. Using computational modeling and quantum mechanical methods, the current study focused on further characterizing the relationship between molecular structure and the energetics of Bergman Cyclization. It was found that the number and type of tautomeric sites did not have an effect on activation barriers and endothermicities. Also, the data showed that the incorporation of the enediyne into a ten-membered ring increased endothermicity and that the addition of two carbonyl groups to the ten-membered ring system caused activation barriers and endothermicities to decrease. The theory presented throughout this research utilizes fundamental chemistry principles in order to create a unique solution.

ADVENTURES IN THE INVESTIGATION OF THE THERMAL DECOMPOSITION OF METAL CARBONATES. T. C. DeVore, Rafael Snell-Feikema, & Neil Mehta, Department of Chemistry and Biochemistry, James Madison University, Harrisonburg VA 22807. The thermal decomposition of magnesium oxalate differs from the other group 2 oxalates in that a stable magnesium carbonate intermediate (MgCO<sub>3</sub>) is not observed during the decomposition process. Attempts to establish the thermal stability of MgCO<sub>3</sub> was thwarted when commercial samples of "MgCO<sub>3</sub>" from two different suppliers (Acros and Fisher) were identified as

hydromagnesite  $(Mg_5(CO_3)_4(OH)_2-4H_2O)$  using Attenuated Total Reflectance – FTIR and powdered X-Ray diffraction. The thermal decomposition under normal conditions occurred in two steps. The waters of hydration were lost between 450-550 K to form  $Mg_5(CO_3)_4(OH)_2$  which then decomposed between 600-800 K to produce MgO. However, if the pattern changed drastically when experimental conditions were changed to hider the removal of the gaseous products from the cell by placing a top on the cell or increasing the sample size and or the heating rate. Under these conditions, the transition at 700 K split into two partially resolved transitions and a new transition above 800 K was observed. It is hypothesized that the increased pressure of  $CO_2$  in the cell slows the decomposition of the amorphous MgCO3 phase produced by the loss of water from  $Mg_5(CO_3)_4(OH)_2$  allowing it to be observed. If stabilized enough, this phase is converted to a new phase at 808 K. The decomposition of this phase produced the transition above 800 K.

# **Posters**

CONSERVATION STUDIES OF GB1 SEQUENCE AND STRUCTURE. J. Bedford, J. Collins, & L. Greene, Dept. of Chemistry and Biochemistry, Old Dominion University, Norfolk, VA 23529. The objective of this project was to perform a bioinformatics study of the immunoglobulin G-binding domain 1 of Streptococcal protein G (GB1) as well as a biophysical analysis of the wild-type protein. The hypothesis of this experiment proposes that evolutionarily conserved residues are key for the formation of protein structure. The bioinformatics investigation was carried out using the Dali-Lite server to find proteins from a wide range of organisms that retain a similar structure but have different functions. Of the proteins found thirteen different proteins were selected from various species. Conservation analysis was performed and showed that twelve of fifty-six positions are highly conserved. Hydropathy analysis was performed and showed that eight of the twelve residues are hydrophobic. A threedimensional image shows that all eight of these hydrophobic residues could be in the core of the protein. To investigate the importance of these conserved residues a characterization of wild-type GB1 using biophysical techniques must be performed. The biophysical studies that were carried out were near- and far-UV circular dichroism, equilibrium fluorescence, and stopped-flow fluorescence. These techniques specifically allow for the characterization of GB1 under a specific set of conditions which will be used as a reference when mutations are done in the future.

THE INFLUENCE OF SYNTHESIS ENVIRONMENTS ON NANOPARTICLES. Perrin Godbold<sup>1</sup>, Brigitte Wendell<sup>1</sup>, Kiara Pontious<sup>2</sup>, Hilary Benedict<sup>2</sup>, Gail Moruza<sup>2</sup>, & Dr. Kyle Gipson<sup>2</sup>, <sup>1</sup>Dept. of Chemistry and <sup>2</sup>Dept. of Engineering, James Madison University, Harrisonburg VA 22807. In the field of optical materials, polymers are studied as an alternative to silica glass, which is currently the dominant matrix. Polymers have the advantage of being lighter than silica glass, but have issues with attenuation caused by high vibrational energy. Attenuation limits the effectiveness of the fiber optic cables and reduces the emissions of the rare-earth ions if dispersed without a protective polymer. To reduce attenuation, fluorescent nanoparticles are added into the polymer. This research explores the factors that impact the luminescent properties of rare-earth ions when the rare-earth ions are a part of low vibrational

energy structure such as a nanocrystal within the polymer matrix. Rare earths ions Terbum<sup>3+</sup> and Europium<sup>3+</sup> (Tb<sup>3+</sup> and Eu<sup>3+</sup>) are doped into Lanthanum Fluoride (LaF<sup>3+</sup>) to form light emitting nanoparticles and then combined with acetylsalicylic acid, (an organic ligand which aids in dispersing the nanocrystals within the polymer matrix). This research focuses on varying synthesis environments for rare-earth doped nanoparticles. The main objective is to analyze the composition of the nanoparticles to determine the effects of the various synthesis environments on the luminescent properties, size and distribution of the agglomerates. This part of the project has looked at the characterizations of four samples of nanoparticles using a scanning electron microscope (SEM), electron dispersion x-ray spectroscopy (EDX), power x-ray diffraction (PXRD) and a rheometer.

BONDING PATTERNS OF PHENALENE AS A LIGAND BONDED TO METALS. Ting Li, Huiling Shao, Alan DeLorenzo, Emma W. Goldman, & Donald Kelling, Department of Chemistry, University of Richmond, Richmond, Virginia 23173. Phenalene is an interesting organic compound with three fused rings. This project investigates the bonding patterns of phenalene as a ligand bonded to metals. Results of a theoretical study will be presented that indicate that the shape and structure of the computed sandwich metal complex modify dramatically along the periodic table. We will discuss our results thus far in our synthesis of the interesting transition metal compounds.

OXIDATION OF HISTIDINE AND CARNOSINE USING HYPOCHLOROUS ACID. A. B. Nzesi, S. Babu, & M. O. Claville, Department of Chemistry, School of Science, Hampton University, Hampton, VA 23668. Peptides and proteins are prone to oxidation by means of reactive oxygen species such as hypochlorous acid (HOCl). These oxidized substrates may promote disease in different organs. Hypochlorous acid is a powerful oxidant that is produced and utilized by the body during the immune response. The presence of an imidazole ring in histidine and carnosine make these biomolecules (amino acid and dipeptide, respectively) instrumental in protecting against oxidative damage. Our interest in the role of these two molecules in defending against oxidative damage, has led us to investigate the mechanism of chemical oxidation with biochemical oxidants, Reactions were performed with 0 to 4 µmol of hypochlorous acid in 2 mL of 0.1 M phosphate buffer, pH 7.0, and 0.1 mM DTPA, while keeping the concentration of histidine or carnosine (2 µmol) constant. All reactions were monitored using a UV-Visible spectrophotometer. Both histidine and carnosine were oxidized in the presence of HOCl. In the case of histidine, UV data showed a decrease in the absorbance at 292 nm and an additional peak at 210-230 nm, with increasing concentration of oxidant. Interestingly, carnosine showed spectral shift from 292 nm to 300 nm with increasing concentration of HOCl. This absorption increased with increasing concentration of oxidant, suggesting the formation of a new species. [Supported by the National Science Foundation-Faculty Early Career Development (CAREER) Award, CHE-0847742 and ACE Implementation Award NSF HRD-1238838]

SYNTHESIS OF DICYSTEINE AND DIMETHIONINE PEPTIDES. <u>D. Oliver</u>, S. Babu, & M. O. Claville, Department of Chemistry, School of Science, Hampton

University, Hampton, VA 22668. Dipeptides Methionine-Methionine (Met-Met) and Cysteine-Cysteine (Cys-Cys) were synthesized using solid-phase peptide synthesis with an automated peptide synthesizer (Tribute, Protein Technologies, Tempe, Arizona). The syntheses were executed from C- to N-terminus, and utilized the 9fluorenylmethyloxycarbonyl (FMOC) protecting group to prevent polymerization of the N-terminus of the amine group (Scheme 1). The syntheses involved the following consecutive steps: (1) swelling of the appropriate resin containing the C-terminal amino acid; (2) deprotection with 20% piperidine; (3) washing with dimethyl formamided (DMF); (4) coupling with the second amino acid using HBTU (O-(Benzotriazol-1-yl)-N,N,N,N -tetramethyluronium hexafluorophosphate), or HCTU(2-(6 - Chloro - 1H - benzotriazole - 1 - yl) - 1,1,3,3 - tetramethylaminium hexafluorophosphate) in the presence of N-methylmorphine; removal of the dipeptide form the resin; and (5) washing of the product. The crude products were analyzed using Delta Jeol NMR (400MHz) and Infrared Spectrophotometer (Varian 1000 FT-IR). Future directions for this project involve the optimization of the syntheses followed by oxidation with peroxynitrite. [Supported by the National Science Foundation-Faculty Early Career Development (CAREER) Award, CHE-0847742 and ACE Implementation Award NSF HRD-1238838]

POLYELECTROLYTE MULTILAYER FILMS: UV-VISIBLE STUDIES OF METHYLENE BLUE LOADING AND RATWES OF RELEASE. Alison M. Washington, & Kevin W. Kittredge, Department of Chemistry, Virginia Wesleyan College, Norfolk, VA 23502. A study of the rates of release of methylene blue (MB) from hyperbranched poly(acrylic acid)/poly(allylamine hydrochloride) (PAA/PAH) films at 38 °C and pH 7.15 is reported. Hyperbranched were synthesized in a layer-by-layer fashion with an intercalated organic dye, methylene blue, and cured at 150 °C for 15 min. The films were approximately 575 Å in thickness as measured by profilometry. The rates of release were measured by UV-visible spectroscopy by monitoring the appearance of methylene blue at 665 nm. Two processes have been observed, the first is for the release of methylene blue from the film and the second, slower process is undetermined. We are examining two potential processes to determine which corresponds to the slower rate, hydrolysis of the free methylene blue in solution or decomposition of the film.

RATIONAL DESIGN OF CHEMICAL INHIBITORS FOR PROTEIN N-TERMINAL METHYLTRANSFERASE. Gang Zhang, Yunfei Mao, Stacie Lynn Richardson, & Rong Huang, Department of Medicinal Chemistry and Institute for Structural Biology and Drug Discovery, Virginia Commonwealth University, Richmond VA 23219. The objective of this study is to develop potent inhibitors of N-terminal RCC1 methyltransferase (NRMT). NRMT is upregulated in a variety of cancers and plays an important role in regulating protein-DNA binding. Depletion of NRMT increases multipolar spindle formation during cell mitosis. Thus, NRMT plays an important role in cell growth and may be a potential anticancer target. However, there is no chemical tool available to characterize NRMT function. Hence, we aim to develop potent inhibitors for NRMT, which can be applied as chemical probes to validate the role of NRMT function in cancer cell growth and as lead compounds to develop novel anticancer therapeutic agents. We used a mechansim-based approach to design NRMT inhibitors

to mimic the transition state. They have three components: an N-adenosyl methionine (NAM), a peptide derived from N-terminal protein substrate, and a linker. We adopted a fluorescence-based assay to evaluate the activities of bisubstrate analogs. We have successfully synthesized a series of bisubstrate analogues and determined their inhibitory activities. Among them, SPK-NAM and SPKR-NAM have an IC 50 of 51.9 and 13.4 uM, respectively. As negative controls, both NAM-triazole and triazole-peptide did not show any significant inhibition at 100 uM. We have synthesized the first NRMT inhibitors using a mechanism-based strategy. These inhibitors showed inhibitory activities in the micromolar range. These results suggest the possibility of developing potent NRMT inhibitors using a bisubstrate approach.

# Computer Science

ADOPTING SECURITY ASPECTS INTO THE SOFTWARE DEVELOPMENT LIFE CYCLE. Yen-Hung Hu, Department of Computer Science, Hampton University, Hampton, VA 23608. Our research has found that security vulnerabilities exist in most undergraduate capstone and programming projects that are mainly caused by ignorance of security aspects in coding. This raised our concern about adopting security aspects into the teaching of the software development life cycle in undergraduate software engineering and programming courses. To emphasize this concern, we developed a security assessment table to identify vulnerabilities and security breaches in such projects. The assessment table consists of 13 critical questions which are derived from several prominent institutions including Oracle, CERT, and CMU. We also adopt three security characteristics: confidentiality, integrity, and availability, from the McCumber Cube framework, as the measurement criteria for each critical question in the assessment table. For every captured security vulnerability and breach, we identify solutions that follow Oracle and other secure coding guidelines.

FROM SCRATCH TO JAVA. Bruce Chittenden, Department of Computer Science, Hampton University, Hampton VA. Our high schools are failing us because students entering college today have few courses in Computer Science. Many students have no idea what Computer Science is about or think Computer Science is about creating video games. Most students also lack critical thinking and problem solving skills which are essential to Computer Science. For the past three years, we have used the Scratch programming language in an entry-level course to introduce students with no background to Computer Science. Scratch is a 2-dimensional drag-and-drop Lego-like programming language from the Lifelong Kindergarten Group at MIT. Students spend a semester learning the concepts of programming languages through Scratch. The final project for this course is to create a Scratch Program that implements a Children's Story. This year we created a series of fifteen labs that demonstrated most of the programming constructs of the C-based Programming Languages (specifically, Java). The labs are structured so that the student first writes the program in Scratch, make sure it works, and then writes the same program again in the Java. Since Scratch gives a visual representation of the program, students can more easily visualize the program when implementing it in Java. The titles of the fifteen labs are listed below to show the various programming constructs that are presented in this approach: "Hello World!",

"Reading Keyboard Input", "Addition and Subtraction", "Multiplication and Division", "If", "If Else", "Nested If", "Logical Operators", "Random Numbers", "For Loop", "While Loop", "Nested Loop", "Function Calls", "ArrayLists", and "Arrays."

#### Education

THE SPEED EXHIBITION. C. Mills<sup>1</sup>, E. G. Maurakis<sup>2</sup>, R. Conti<sup>2</sup>, L. Hughes<sup>2</sup>, & D. Hagan<sup>2</sup>, <sup>1</sup>College of William & Mary, Williamsburg VA 23187 and <sup>2</sup>Science Museum of Virginia, Richmond VA 23220. Objectives of the 10,000 ft<sup>2</sup> exhibition gallery project include creating interactive exhibit experiences to challenge visitor's assumptions, changing their perceptions about the universe and themselves, gaining fascinating scientific insight, and reflecting on their own or with others so they are better prepared to have an informed opinion of the world around them. The SR-71 Blackbird aircraft, suspended over the exhibition, will serve as the icon for the Speed exhibition. Front-end evaluation informed SMV personnel of the understanding level of the concept of speed by visitors as well as strategies to convey topics. Most visitors associated the concept of speed with fast change over time, and did not consider evolution and geologic processes as such. However, after being presented the concept of change over long periods of time, visitors acknowledged that both evolution and geologic processes fit the concept of speed. Over 40 interactive exhibits have been designed to create an atmosphere of wonder by communicating STEM concepts in ways that are insightful and assured as well as surprising and quirky. The overarching theme is change over time, a perspective crucial for understanding scientific concepts - from the speeds of light and sound, expansion of the universe, NASCAR racing vehicles, technology, telecommunications, robotics, and growths in human population, plants, and social media to geological changes, evolution, and speciation.

PROMISING PARTNERSHIPS AND PEDAGOGY FOR AFTER-SCHOOL SCIENCE. Charles L. English, Dir. Playful Learning and Inquiry, Science Museum of Virginia, Richmond VA 23220. The Science Museum of Virginia is a center for scientific investigation, building curious minds, inspiring people to dig further into science, or STEM, and how it impacts our daily lives. The Museum's Mission -Inspiring Virginians to enrich their lives through science - is designed to engage inquiring minds through rich experiences. The social climate has been slowly shifting within the past few years - to address an area that does not get enough attention - to inspire the minds of youth that may otherwise turn away from a scientific endeavor to grow a STEM Pipeline towards STEM Careers. To this end the Science Museum is evolving. Our goal is not to be a repository for historic science content as much as to evoke inspiration, enthusiasm, and exploration. Learn about the outreach program successes, areas of need, uncovering stories and experiences as we all look to advance our learning community, especially in an informal, after-school setting. Our current partners include Boys & Girls Clubs of Metro Richmond, Higher Achievement, YMCA, 4-H, Communities in Schools, Richmond Public Schools and the Virginia Library Association. The pedagogical approach is aligned with project based or problem based learning which encourages youth to use their own voice and choice in solving task given to them through effective use and implementation of digital media.

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PLUMBING THE STEM PIPELINE. Megan K. Healy, Virginia Director of STEM, Commonwealth of Virginia, Richmond VA 23219. Named the best state for STEM jobs, Virginia can only maintain this status if a robust, 21st century workforce is produced from the P-20 education and workforce systems. To strengthen this "pipeline", students need to be excited in elementary school, inspired in middle school and gain foundational skills in high school to go to college or into the workforce. Many leaks and exit points are found in the current system. Not enough students are interested in STEM and if they are, they do not graduate from STEM programs, Virginia STEM is working with businesses and educators to increase the number of globally competitive Virginians to meet the market demands. The goals of Virginia STEM are to inspire the building of a strong STEM workforce and education pipeline, integrate business and education efforts, initiate communication among all stakeholders and innovate promising practices that can be replicated or scaled. With improved coordination at the state level, Virginia will be a global leader in STEM by providing outstanding education and career opportunities.

## Posters

RESOURCE USE BY COLLEGE FRESHMEN IN A FIRST-SEMESTER HUMAN ANATOMY AND PHYSIOLOGY COURSE. Lisa D. Williams, Math, Science and Eng. Div., No. Va. Comm. Coll., Annandale, VA 22003. Students (N=56) in a first-semester human anatomy and physiology course were polled regarding resources used to study for their first lecture exam. Students overwhelmingly reported (91%) using class notes they generated as their primary study resource. More than half the students used the assigned lab manual (64%) or course textbook (58%) when studying. The percentage of students reading course textbooks approximates that of psychology students in another study. Students also formed study groups and utilized books and models in a campus Science Learning Center. Future assessments could determine how often, and how extensively, students are reading and using other study tools, including study guidelines.

STM 101: A NOVEL TEAM TAUGHT UNIVERSITY/HIGH SCHOOL ENTERPRISE, PHASE II. H. A. Rowe<sup>1</sup>, M. E. Anderson<sup>1</sup>, C. E. Bonner<sup>1</sup>, J. D'Silva<sup>1</sup>, A. Fernando<sup>1</sup>, D. Geddis<sup>1</sup>, C. Hinds<sup>1</sup>, M. O. Keeve<sup>1</sup>, H. Kinlaw<sup>1</sup>, L. Salary<sup>1</sup>, P. M. Konopnicki<sup>2</sup>, & S. L. Sutton<sup>2</sup>, <sup>1</sup>Norfolk State University, Norfolk, VA 23504 and <sup>2</sup>Virginia Beach Public School System, Virginia Beach, VA 23456. NSU is in the second year of a partnership with VBPS to teach a novel course: STM 101. Qualified HS students received elective credit for completing this course which involves separate team taught modules on Comp. Sci, Chemistry, Physics, Biology, Technology, Engineering, and Math. This course was taught from a central location remotely to the students in their high schools. The purpose of the program was to give the students a "taste" of the various STEM disciplines and to inform the students about STEM topics

and careers and college in general. The aim was for these students to consider college as an option and specifically a STEM discipline as a major. The modules were presented by enthusiastic faculty using demonstrations, laboratory exercises, and hands-on activities to stimulate the students. Participants were administered a questionnaire to assess this program. Students indicated that due to this class they are more likely to attend college and have a more favorable attitude towards pursuing a major in a STEM field. While the students, and high school student mentors are more satisfied with the Phase II remote delivery of this course, NSU faculty indicates it is still a problem. Both high school and NSU faculty indicate that getting the students to turn in assigned work is a problem. These perceptions have not changed significantly from Phase I. Changes to address these concerns are being planned.

#### **Environmental Science**

MICROBES IN COMPOST. Katherine B. Mireles & Carolyn Thomas, Ferrum College, Ferrum, VA. The purpose of this project was to determine the effects of composts age on the microbial composition of compost. Four compost samples were collected from the composting bins at Ferrum College in October 2013 and January 2014. The samples encompassed the various stages of the composting process ( mesophilic, thermophilic, and curing). The actual temperature of the samples ranged from 0-39°C. The temperature of the compost was believed to have a large impact on the microbial composition. Fungi genera (Aspergillus, Penicillium and Mucor) were similar in both samples collected in October 2013, Similarly the bacteria genera, (Staphylococcus and Bacillus) were similar in the two October samples. When comparing the samples from the two collection dates, there were more differences in the variety of species of both bacteria and fungi. In January there were no cocci shaped bacteria present in any of the samples collected. Bacillus was the most commonly found genus of bacteria found with three out of 15 bacterial colonies isolated being Bacillus. Staphylococcus was the second most common genera found in the samples collected in October with three out 15 isolated bacterial colonies being identified as Staphylococcus.

STATUS OF A CONSTRUCTED WETLAND MITIGATION SITE IN SPOTSYLVANIA COUNTY, VIRGINIA. Michael L. Bass, Robert Ericson, Zack DelGrosso, & Rebecca Conway, Dept. of Earth & Environmental Sciences, University of Mary Washington. The purpose of this project was to analyze the health of two streams that run through Spotsylvania County, Virginia, Massaponax Creek and the Ni River. While Massaponax Creek is an urban tributary and flows through highly developed residential, business, and commercial areas, the Ni is an example of a more rural territory. Together, the streams illustrate the effects of urban development. Massaponax Creek has four sampling locations, while the Ni River has three. For each site, pollution levels were implied through a combination of macrobenthic sampling and water chemistry analyses such as phosphate and nitrate concentrations, total suspended solids and total dissolved solids, and fecal coliform levels. The results were compared to previous research to best identify how stream health changes over seasons. According to the macrobenthic results in the fall, nearly all sites saw an increase in

%EPT, except for Massaponax 208 and Ni Route 1, which fell from 80% to 54% and 88% to 78%, respectively. This improvement in the majority of Massaponax Creek %EPT may be a result of an increased proportion of *Trichoptera*, a more resilient macrobenthic invertebrate, while the Ni River experienced an overall increase in *Ephemeroptera*, which are less tolerant of pollutants. Although both Massaponax Creek and the Ni River are under legal limits for phosphate and nitrate concentrations, the phosphate levels are usually higher in summer on the Massaponax and winter for the Ni, while nitrates peak during the winter and spring for both streams. TDS rose during the summer and then declined, as precipitation rates fell. Massaponax Creek has higher TDS than the Ni, but that is reversed for TSS. Fecal coliform levels greatly decreased from summer into winter and fall, with both streams remaining under legal limits. The overall health of both streams has greatly improved, as suggested from the increased %EPT. This speaks to the recovery of the streams, resulting from repaired leaking sewer lines and decreased erosion risks from buffer zone stability in surrounding areas specifically near Massaponax Creek.

OYSTER RESTORATION ON THE HALF SHELL. Todd. V. Janeski, Center for Environmental Studies and Rice Center, VCU, Richmond, VA. The VCU Rice Rivers Center confirmed the proof of concept (POC) to collect business generated oyster shells for the purpose of enhancing the Eastern Oyster (*Crassostrea virginica*) at sanctuary sites in the Virginia portion of the Chesapeake Bay. This successful pilot lead by the VCU Rice Rivers Center brought together private businesses, state and local government and nongovernmental organizations to collect nearly 12,000 lbs. of shell in four months. The POC was essentially a zero-budget approach to demonstrating a coordinated effort developing a single vision, to collect and return used oyster shell to the Virginia portion of the Chesapeake Bay for the purpose of wild oyster restoration. Through the demonstrated POC, the VCU Rice Rivers Center advanced the pilot into a Program where more than two dozen public, private and NGO partners are working to enhance restoration efforts. Non-traditional partnerships and outreach activities are effective strategies to engage a broad range of partners and the general public to become involved in Chesapeake Bay restoration programs.

SPATIAL MULTILEVEL MODELING WITH FUNCTIONAL COEFFICIENTS: APPLICATION TO CONOPY VEGETATION PERCENTAGE OF PLOUR RANGELAND, IRAN. Hossein Moradi Rekabdarkolaee, Dept. of Statistical Sciences and Operations Research, VCU, Richmond, VA. 23284. In spatial data analysis, data sometimes are nested in different groups. Hence, beside the spatial correlation, intraclass correlation is another type of correlation in the data. Considering this correlation in the structure of modeling brings us to a multilevel model. Such models provide an appropriate framework for the dependent data which are collected at different levels. In spatial multilevel models the coefficients can be functions of the other variables which lead us to functional model. In this paper we propose a spatial multilevel model with functional coefficients and in order to present the statistical inference, the Bayesian approach is utilized to analyze the model and illustrate its application in zoning of Plour's rangeland canopy vegetation percentage. Examining the proposed model, it is found to provide satisfactory results in compare with the classical

multilevel model, traditional spatial model, and multilevel model with spatially varying coefficients.

THE EFFECT OF WATER LEVELS ON CARBON DIOXIDE EMISSIONS FROM SOIL MICROCOSMS IN THE GREAT DISMAL SWAMP. Justin L. Weiser, Dept. of Orgamisal and Environmental Biology, CNU, Newport News, VA. Peatlands contain large amounts of stored carbon and form where soils are saturated for long periods. Anthropogenic disturbances can lower water tables and reverse dissimilation and sequestration functions. This study modeled the effect of restored and unrestored hydrology on carbon respiration at two soil depths. Sites are located in northeastern North Carolina within the Dismal Swamp State Park, Carbon dioxide (CO<sub>2</sub>) efflux was measured in 24 cores at approximately 30-cm and 60-cm increments below the surface. Microcosm simulations were conducted for 61 days beginning with unrestored (31 days) and then restored hydrology (30 days). Efflux of CO<sub>2</sub> in unrestored microcosms  $(1.76 \pm 0.07 \,\mu\text{mol CO}, \bullet \,\text{m}^{-2} \bullet \,\text{s}^{-1})$  was greater than in restored microcosms  $(0.62 \pm 0.3)$ μmol CO<sub>2</sub> • m<sup>-2</sup> • s<sup>-1</sup>). As a result, modeled annual emissions of CO<sub>2</sub> were significantly greater in unrestored (13.37 t C • ha<sup>-1</sup> • yr<sup>-1</sup>) than restored (3.7 t C • ha<sup>-1</sup> • yr<sup>-1</sup>) simulated conditions, suggesting that carbon sequestration benefits may be derived from raising water levels in the Dismal Swamp. Supported by: Sigma Xi Grant-in-Aid of Research.

### Posters

NUTRIENT EXCRETION BY FRESHWATER SNAILS: EFFECT OF FEEDIG AND BODY MASS. <u>David McGuire</u> & Maynard Schaus, Dept. of Biology, VWC, Norfolk, VA 23505. Consumer-mediated nutrient cycling can have an impact on the chemical composition of the environment. Nitrogen and Phosphorus are potentially limiting resources in freshwater habitats. As such, the rates of excretion of N and P by animals may be important to aquatic primary producers. In order to determine the factors that influence the rate of nutrient cycling, the levels of phosphate and ammonia excreted by pond snails were examined based on the sizes of snails and whether or not they had recently been fed. No significant effect was found for the differences in size between snails and the rate of excretion, but this study did conclude that undernourished snails excreted almost no phosphates, and only a small amount of ammonia. Therefore, the snail's contribution to the environmental levels of these compounds is reliant on the amount of food they eat.

MERCURY CONCENTRATIONS IN DOLPHINS STRANDED ALONG THE COAST OF VIRGINIA. E. Smith, E. Malcolm, R. Ellick², K. M. Phillips³, & M. Lynott³, Dept. of Earth and Environmental Sciences, VWC, ²Institute for Integrative Bird Behavior Studies, WM, ³Virginia Aquarium Stranding Response Program. This study was conducted to assess the mercury levels in bottlenose dolphins (*Tursiops truncatus*) from North Atlantic communities. This is the first study to assess the mercury levels in bottlenose dolphins off the coast of Virginia. Mercury, released from both anthropogenic and natural processes, is a highly toxic metal that bioaccumulates in the tissues of top marine predators, including dolphins. As a result, tissue concentrations of dolphins from Atlantic communities can be used an as indicator of relative mercury concentrations throughout their corresponding food webs. Tissues

were subsampled from 19 North Atlantic bottlenose dolphins collected by the Stranding Response Team of the Virginia Aquarium. This included a total of 18 liver samples, 13 kidney samples, two skin samples, and two muscle samples. The samples were freeze dried and then analyzed for total mercury using a DMA-80 at the College of William and Mary. Liver samples were found to contain the highest levels of mercury, followed by the kidney samples. The lowest concentrations were found in skin and muscle samples. There were no significant differences in concentration between sexes, though there was a positive correlation between length and mercury concentrations in the liver and kidney. The concentrations in these dolphins are most likely related to their age and their diet.

CITIZEN SCIENCE! A NOVEL APPROACH TO ASSESS THE IMPACTS OF URBANIZATION ON VIRGINIA BIRDS. Emily Crawford, Brady P. Donovan, Sujan M. Henkanaththegedara & Mark L. Fink, Department of Biological & Environmental Sciences, LWU, Farmville VA 23909. It has been shown that bird diversity has changed in response to urbanization. However, most previous studies have been narrowly focused and limited to localized study sites. We studied the statewide impact of urbanization on Virginia birds using a citizen science data base, eBird supported by the Cornell Lab of Ornithology and National Audubon Society. We assessed the impacts of urbanization on Virginia birds by comparing, 1) species richness, and 2) feeding guild composition between urban areas (cities/towns) and natural areas (state parks). Natural areas (N = 34, average richness = 115.18) had a significantly higher (t = 5.659, P < 0.001) species richness compared to urban areas (N = 34, average richness = 52.12). However, we failed to detect any significant differences of feeding guild composition between natural and urban areas for 6 feeding guilds of birds (N = 10; d.f. = 1; F = 0.959; p = 0.226). Our work shows the feasibility of utilizing citizen science data bases to assess the impacts of urbanization on wildlife populations covering large geographic areas. Future research involves expanding the analysis to the southeast United States to understand continental scale patterns.

EFFECT OF SEX ON NUTRIENT EXCRETION IN SEXUALLY DIMORPHIC FISHA. Lisa Murray & Maynard Schaus, Dept. of Biology, VWC, Norfolk, VA 23505. Previous research on nutrient cycling by fishes has examined factors such as mass, temperature, and dict, which can have important effects on nutrient excretion. However, no previous study has examined the effect of sex on nutrient excretion, even though sex can have large impacts on fish size and reproductive investment. This study was undertaken to determine whether the sex of a fish impacts the excretion of nitrogen and phosphorus in Betta splendens and Poecilia reticulata. It was hypothesized that nutrient excretion would be significantly impacted by both mass and sex. We conducted direct nutrient excretion measures by examining changes in N and P concentrations over a 4 hour period. We detected no significant differences in excretion rates of males and females for B. splendens or P. reticulata. Ammonia nitrogen excretion was significantly higher in the fed fish treatment of B. splendens, compared to the unfed treatment. In both species, we detected no significant effect of mass, likely because we restricted our measures to adult fish of similar size. Our results indicate that sex is not likely important to nutrient cycling rates. Feeding history can have important impacts on excretion rates, especially for nitrogen.

EFFECTS OF BODY MASS AND TIDAL SUBMERGENCE ON NITROGEN EXCRETION IN THE RIBBED MUSSEL, Geukensia demissa, Jeff Illinik<sup>1</sup>, Raluca Illinik<sup>2</sup> and Maynard Schaus<sup>1</sup>, <sup>1</sup>Dept. of Biology, VWC, Norfolk, VA 23505 and <sup>2</sup>Dept. of Molecular Biology & Chemistry, CNU, Newport News, VA 23606. Geukensia demissa is an abundant filter feeder that may have important effects on nutrient cycling within East Coast salt marshes. This species is thought to interact mutualistically with salt marsh cordgrass (S. alterniflora), as nutrient cycling by mussels fertilizes the cordgrass, which provides attachment sites for mussels. We investigated the effects of mussel mass and tidal immersion on nitrogen excretion by G. demissa in the Lafayette River, Norfolk, VA. Mussels were collected, cleaned, and placed in bags containing filtered estuarine water, which was later analyzed for total dissolved N using 2 point UV spectroscopy following persulfate digestion. We observed a significant negative correlation between mussel mass and mass-specific N excretion, N excretion remained consistently high through the tidal cycle, unlike P, which was previously shown to decrease dramatically after 1.5-3 hours of tidal immersion. This consistent release of N has important implications for cordgrass productivity, especially in the outer marsh zone where G. demissa is abundant.

EFFECT OF URBANIZATION ON SALT MARSH BIRD DIVERSITY AND COMMUNITY COMPOSITION. Maynard Schaus & Mark Petersen, Dept. of Biology, VWC, Norfolk, VA 23505. The impact of urbanization on salt marsh bird communities was investigated in the Lynnhaven River estuary, Virginia Beach, VA during summer 2012-2013. Bird diversity, abundance, and community composition was compared across 18 sites grouped into 4 urbanization categories (low, medium, high, and very high). The intermediate disturbance hypothesis predicted the highest diversity and abundance in areas of moderate disturbance, in this case the medium to high urbanization categories. However, diversity and abundance did not differ significantly with urbanization. Bird community structure differed significantly with urbanization, as Great Blue Heron and Purple Martin were more abundant in areas with low urbanization, and generalist species (e.g., Laughing Gull, Common Grackle, Herring Gull) dominated in areas with very high urbanization. This shift in bird community structure is similar to the type of shift observed in forest bird communities across a gradient of urbanization. However, unlike forest bird communities, the intermediate disturbance hypothesis was not supported.

COMPARATIVE STUDY OF WATER QUALITY PARAMETERS IN NI RIVER AND MASSAPONAX CREEK IN SPOTSYLVANIA COUNTY, VIRGINIA. Robert K. Ericson, Zack DelGrosso, Rebecca Conway, & Michael L. Bass, Dept. of Earth & Environmental Sciences, University of Mary Washington. The goal of this research was to determine the health of two Spotsylvania County streams, the Ni River and Massaponax Creek, in Virginia. The streams illustrate the effects of urbanization, since the Ni River is a rural tributary and Massaponax Creek travels through a more developed area. At four sites on Massaponax Creek and three on the Ni, macrobenthic samples were taken as bio-indicators of pollution. Water chemistry testing then supported this analysis, such as calculating phosphate and nitrate concentrations, total suspended solids and total dissolved solids, and fecal coliform levels. Comparisons between the two streams across different seasons illustrate how the overall health of

each stream ecosystem may be impacted by land use and intensified human expansion. According to the fall macrobenthic results, nearly all sites saw an increase in %EPT, except for Massaponax 208 and Ni Route 1. Even though both Massaponax Creek and the Ni River are under EPA legal limits for phosphate and nitrate concentrations, the phosphate levels usually rose in summer on the Massaponax and winter for the Ni, while nitrates peak during the winter and spring for both streams. TDS increased during the summer and then declined, as precipitation rates fell. Massaponax Creek has higher TDS than the Ni, but that is reversed for TSS. Fecal coliform levels greatly decreased from summer into winter and fall, with both streams remaining under legal limits. This speaks to the recovery of the streams, resulting from repaired sewer lines and decreased crosion risks in surrounding areas specifically near Massaponax Creek. Due to the majority increase in %EPT, decreased concentration of nitrates and phosphates, and reduced fecal coliform levels, the Ni River and Massaponax Creek have generally seen an improvement in overall health.

## **Medical Sciences**

THE ACTIVATION STATUS OF RHEB IN PEMETREXED TREATED HUMAN CANCER CELLS. C. M. Bell, S. Agarwal & R. G. Moran, Dept. of Pharmacology & Toxicology, Virginia Comm. Univ., Richmond, VA 23298. Rheb (Ras homolog enriched in brain) is a small GTPase essential for activating mTORC1 (mammalian target of rapamycin complex 1) when loaded with GTP, mTORC1 activation increases cell growth by promoting protein synthesis and lipogenesis. Tuberous sclerosis protein 2 (TSC2) negatively regulates mTORC1 by stimulating the GTPase activity of Rheb. Pemetrexed (PTX) is a multi-targeted antifolate drug that suppresses tumor growth by obstructing folate metabolism and indirectly activating AMP activated kinase (AMPK). AMPK-activators inhibit cell growth by activation of TSC2 and deactivation of the mTORC1-partner protein, Raptor. We have shown that PTX treatment does not increase the activating phosphorylation of TSC2, but that mTORC1 activity is still significantly decreased. Further, we have also shown that in cells null for p53, more Rheb is associated with Raptor and mTORC1 activity is increased. Thus, two methods have been developed to measure the levels of Rheb-bound nucleotide. Coupled enzymatic assays using nucleoside diphosphate kinase were employed to convert GTP to ATP, and the ATP quantified in a luciferase assay. Corresponding GDP was converted to  $\gamma^{32}$ P-GTP and quantified by reverse phase-HPLC and liquid scintillation counting. Alternatively, cells were metabolically labeled with <sup>32</sup>P orthophosphate and the Rheb-bound nucleotides resolved by TLC. These two methods show the levels of Rheb-GTP to be increased in p53 null cells and surprisingly slightly increased in AICAR or PTX treated cells. Further studies aim to elucidate these data, specifically by assaying subcellular compartments of the cell for Rheb activation.

TOWARDS UNDERSTANDING MOLECULAR INTERACTIONS OF ETHYLENEDIOXY COUNTERPARTS OF MDMA AND METHYLONE AT hDAT. F. T. Sakloth<sup>1</sup>, F. Del Bello<sup>1</sup>, R. Kolanos<sup>1</sup>, J. Partilla<sup>2</sup>, P. D. Mosier<sup>1</sup>, M. H. Baumann<sup>2</sup> & R. A. Glennon<sup>1</sup>, <sup>1</sup>Department of Medicinal Chemistry, VCU, Richmond, VA 23298 and <sup>2</sup>National Institutes of Health, NIDA, Baltimore, MD 21224. MDMA ("Ecstasy")

and its β-keto analog, methylone (MDMC), are controlled (Schedule I) substances that are popular recreational drugs. They act as releasing agents at dopamine and/or serotonin transporters (DAT and/or SERT). The ethylenedioxy counterparts of MDMA (EDMA) and MDMC (EDMC) are found on the "street" and could represent a potential abuse problem, but there is little information regarding their actions. The goals of this project are to 1) examine these ethylenedioxy analogs at DAT using in vitro (synaptosomal) release assays, and 2) construct homology models of hDAT using the crystal structure of D. melanogaster followed by docking studies to determine if differences in potencies might be explained by interactions at DAT. Release data showed that (?)EDMA and (?)EDMC are 8- and 4-fold less potent, respectively, than MDMA ( $IC_{50}$ =75 nM) and MDMC ( $IC_{50}$ = 133 nM) and that S-EDMA ( $IC_{50}$ =276 nM) is 50-fold more potent than R-EDMA, Modeling studies suggest that potentially unfavorable interactions of the ethylenedioxy group of EDMA (especially its R-isomer) with Ser 149 might explain its lower potency at DAT. Additionally, the difference in the potency of MDMA and EDMA, and of S-MDMA and S-EDMA over their lowerpotency R-isomers seems to be explained by the energy penalty for the binding of the ligands at DAT. EDMA and EDMC are unlikely to be as much of an abuse problem as their methylenedioxy counterparts. [Supported by NIH grant DA 033930.]

ALTERATIONS TO THE SYNAPTIC TRANSCRIPTOME IN RESPONSE TO ETHANOL-INDUCED SENSITIZATION. Megan A. O'Brien & Michael F. Miles, Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond VA 23298. It is well established that mRNA can be transported to neuronal distal processes, where it can undergo localized translation regulated in a spatially restricted manner in response to stimulation, potentially playing a role in synaptic plasticity that results in long-term adaptive brain responses. In order to investigate our hypothesis that ethanol behavioral sensitization results, at least in part, from alterations in the trafficking of mRNAs to distal processes, we profiled the synaptic transcriptome utilizing two distinct methodologies: mRNA microarray and RNA-Seq. By using two independent techniques, which employ different molecular principles, we were able to validate data processing schemes as well as differential expression results. We found that there was a significant overlap between these analyses (p-value < 1.0E-16), where 70% of the genes determined by RNA-Seq to be differentially expressed between the synaptic and cytosolic fractions were also deemed significant by microarray analysis. Expression data was then analyzed to identify 229 synaptically targeted candidate genes differentially expressed as a result of ethanol treatment. Examination of gene expression patterns revealed a number of genes whose levels were altered in response to acute ethanol, but habituated in response to repeated, sensitizing ethanol treatment. Functional over-representation analysis revealed enrichment for genes associated with the endoplasmic reticulum and the extracellular matrix. Support provided by NIAAA grants U01AA016667, P20AA017828, and F31AA021035.

INHIBITION OF MONOACYLGLYCEROL LIPASE: THERAPEUTIC POTENTIAL WITH LIMITED CANNABIMEMTIC EFFECTS. <u>B. Ignatowska-Jankowska</u><sup>1</sup>, M. Mustafa<sup>1</sup>, S. Ghosh<sup>1</sup>, M. Niphakis<sup>2</sup>, R. Abdullah<sup>1</sup>, J. Wilkerson<sup>1</sup>, J. Wiley<sup>3</sup>, B. Cravatt<sup>2</sup>, & A. Lichtman<sup>1</sup>, <sup>1</sup> Dept. of Pharmacology & Toxicology, VCU, <sup>2</sup> The Skaggs Institute for Chemical Biology & Dept. Of Chemical Physiology, TSRI, <sup>3</sup>Research Triangle

Institute, RTP. The endocannabinoid 2-arachidonoyl glycerol (2-AG) plays a role in many physiological processes, and it is degraded by monoacylglycerol lipase (MAGL). While increase in 2-AG signaling by preventing its degradation is a promising strategy in treatment of pain, and several other disorders, its function and effects in vivo remain unclear, MJN110 is a novel, selective inhibitor of MAGL that allows for examination of increase in 2-AG, without affecting other endocannabinoid levels. In the present study we tested MJN110 in a murine model of inflammatory pain induced by carrageenan as well as tetrad and drug discrimination assays. MJN110 showed high efficacy and potency to produce anti-allodynic and anti-edematous effects in the pain model [ED50 (95% confidence intervals, C.I.)=0.26 (0.14-0.47) mg/kg]. MJN110 at high doses produced antinociception and increased locomotor actiovity, but did not induce catalepsy or hypothermia, In drug discrimination assay, MJN110 fully substituted for CP55,940, [ED50 (95% C.I.)=0.84 (0.69-1.0) mg/kg], indicating that it produced cannabimimetic interoceptive effects. The antinociceptive effects of MJN110 in the carrageenan assay were 2.4 (1.6-4.4) [potency ratio (95% C.I.)] more potent than in the drug discrimination. MJN110 significantly elevated 2-AG but not other endocannabinoid levels in the brain. Findings are in agreement with in vitro research showing that MJN110 is highly potent and selective MAGL inhibitor in vivo. The results indicate that MAGL inhibition with MJN110 reverses inflammatory pain at lower doses than those that produce cannabimimetic side effects.

THE ABILITY TO MODULATE AND INFLUENCE IMMUNE CELLS TO IMPACT TUMORS IN DIVERSE CONTEXTS. Se W. Jeong & Timothy N. J. Bullock, Department of Pathology, University of Virginia, Charlottesville, VA 22908. Metastatic melanoma has one of the poorest prognoses for patients as the median survival ranges from six to nine months. Nonetheless, there has been significant progress made over the past several years in which immunotherapy have contributed considerably. Celldex Therapeutics has developed a fully human monoclonal antibody which targets CD27 (CDX-1127), a potent costimulatory molecule that can drive lymphocyte proliferation and expansion. CDX-1127 is able to mimic CD70, which serves as the ligand for CD27. The interaction thus creates an appropriate T cell receptor signal to drive T cell activation. There is a significant interest in using CD27 as a therapeutic model as data have shown strong evidence of anti-tumor response and immunity in recent studies. Whereas Celldex administers clinical trials, our immunology laboratory receives patient samples throughout the treatment for flow cytometry analysis of key immunological molecules, identifying B and T cells and to enumerate specific subsets of T cells. One of the more interesting results from CDX-1127 phase I clinical trial is that the MHC class II surface receptor HLA-DR expression increases in majority of the patients, which suggests the immune system is becoming more active upon treatment. In addition, regulatory T cells significantly decreased after the CDX-1127 treatment further implicating the potential for CD27 target therapy for melanoma patients

DIACYLGLYCEROL LIPASE BETA: NEW EVIDENCE FOR INFLAMMATORY AND NEUROPATHIC PAIN RELIEF IN MICE. J. Wilkerson<sup>1</sup>, S. Ghosh<sup>1</sup>, K. Hsu<sup>2</sup>, B. Cravatt<sup>2</sup> & A. Lichtman<sup>1</sup>, Dept. of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA 23298, <sup>2</sup>The Skaggs Institute & TSRI, La

Diacylglycerol lipase (DAGL), the enzyme responsible for Jolla, CA 92037. generation of 2-arachidonylglycerol, represents a component of the endogenous cannabinoid system as a potential therapeutic target to treat pain. As DAGL-B inhibition leads to decreases in lipopolysaccharide (LPS)-induced prostaglandins & proinflammatory cytokines, we hypothesized that inhibition of this enzyme will reverse nociceptive behavior in a mouse model of inflammatory pain. We tested DAGL-β<sup>(-/-)</sup> & wild type (WT) mice treated with the selective DAGL-\(\beta\) inhibitor KT109 in the inflammatory model of intraplantar LPS (2.5 µg) pain. LPS produced robust increases in sensitivity to light mechanical touch, or allodynia, & increased thermal sensitivity, or thermal hyperalgesia in WT mice. Systemic & local intraplantar injection of KT109 reversed LPS-induced allodynia in a time- & dose-dependent manner, DAGL-β<sup>(-/-)</sup> mice displayed reductions in LPS-induced allodynia. KT109 administration into the contralateral paw did not reduce LPS-induced hyperalgesia or allodynia, suggesting that these effects occurred locally. Neither intrathecal nor intracerebroventricular injection of KT109 reversed LPS-induced allodynia, consistent with a peripheral site of action. These findings suggest DAGL-B inhibition reverses the negative sensori-discriminative state often associated with inflammatory pain.

TOLERANCE TO THE RATE-DECREASING EFFECTS OF CP55,940 DEVELOPS IN MURINE INTRACRANIAL SELF-STIMULATION. T. W. Grim, J. M. Weibelhaus, S. S. Negus & A. H. Lichtman, Dept. of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA 23298. Although not currently abused, CP55,940 is a synthetic cannabinoid that possesses many similarities to abused "non-classical" cannabinoids such as CP47,497 and its C8 homolog in terms of increased potency and efficacy relative to  $\Delta^9$ -tetrahydrocannabinol (THC). Thus CP55,940 was selected as an archetypal synthetic cannabinoid to probe the effects of acute and chronic effects of cannabinoids on operant responding for intracranial selfstimulation (ICSS) of the medial forebrain bundle in C57BL6/J mice. Acutely, CP55,940 (0.3-1.0 mg/kg) produced a dose-related significant depression of operant responding for ICSS (ED<sub>50</sub> value (95% confidence limits) = 0.18 (0.12-0.26) mg/kg). The CB1 receptor antagonist, rimonabant (3.0-10.0 mg/kg; s.c.), significantly attenuated the rate-decreasing effects of CP55,940, indicating CB1 receptor mediation. Repeated administration of CP55,940 (0.3 mg/kg, s.c., q.d. for 7 days) resulted in a significant attenuation to the drug's rate-decreasing effects, indicative of tolerance. There were no measurable spontaneous withdrawal effects for CP55,940 under these experimental conditions. Collectively, these data suggest that the abuse liability of synthetic cannabinoids occurs through a distinct mechanism from other drugs of abuse (e.g., cocaine) that act through the classic mesolimbic pathway. Nevertheless, operant responding for ICSS represents a novel approach to investigate the impact of acute and chronic administration of cannabinoids on reward-mediated behavior.

DISCOVERY OF SMALL MOLECULE INHIBITORS OF PSAA, A POTENTIAL TARGET FOR *STREPTOCOCCUS PNEUMONIAE*. Ahmad J. Obaidullah<sup>1</sup>, Hardik I. Parikah<sup>1</sup>, Todd Kitten<sup>2</sup> & Glen E. Kellogg<sup>1</sup>, <sup>1</sup>Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond, VA 23298 & <sup>2</sup>Philips Institute for Oral Health Research, Virginia Commonwealth University, Richmond, VA 23298. Due to development of multidrug resistance in *Streptococcus pneumoniae* over the last few

years, research has begun to define new drug targets for pneumonia therapy. Multiple experiments with *Streptococcus pneumoniae* performed by different research groups have identified a lipoprotein PsaA that is important for pneumonia virulence and is also a promising target for pneumonia therapy. PsaA is a high-affinity manganese (Mn) transporter that is required not only for pneumonia virulence, but also for aerobic growth of *Streptococcus pneumoniae* in serum. We have employed computer modeling and computational chemistry to virtually screen a small-molecule database for inhibition of PsaA function by targeting the metal binding pocket, performing receptor-based virtual screening, molecular docking to identify potential inhibitors of PsaA function, and scoring. We have developed an assay for screening compounds, including the use of a PsaA mutant, testing of multiple compounds, and identification of small numbers of compounds that inhibit *Streptococcus pneumoniae* growth at concentrations less than 20 µM. We will next experimentally test the compounds' effect on Mn uptake and their PsaA dependence. Supported by American Heart Association grant 13GRNT16100010 to TK.

VARIATION IN TIMING OF HEART SOUNDS RELATIVE TO ECG EVENTS IN COLLEGE STUDENTS. Harold J. Grau, Department of Molecular Biology & Chemistry, Christopher Newport University, Newport News, VA 23606. Students in our Human Anatomy & Physiology II lab course performed an activity that included simultaneous recordings of electrocardiograms (ECG) and heart sounds. The two basic heart sounds occur first at the closure of the atrioventricular (AV) valves (S1), and then at the closure of the semilunar valves (S2). AV closing occurs just at the beginning of ventricular systole, which follows the depolarization of the ventricles, indicated by the QRS complex of the ECG. The second sound occurs just after the ventricles begin relaxing (diastole), which follows the repolarization indicated by the T wave of the ECG. Data were collected from about 2 dozen subjects, both male and female, between agcs of 20 - 22 years old. The delay between peak R - S1 ranged from 9 - 55 ms, with most falling into two "clusters" of 22 - 27 ms, and 41 - 47 ms. The delay between peak T - S2 ranged from 69 - 136 ms, with no obvious "clusters". The time between peak R - peak T varied from 227 - 309 ms, and correlated with both the peak R - S1 and peak T - S2 delays. However, the peak R - S1 delay does not correlate with the peak T - S2 delay. The time between the two sounds (S1 – S2) varied from 307 - 382 ms, and correlates positively with the ECG correlates of systole (peak R - peak T).

QSAR STUDY OF 4-SUBSTITUTED METHCATHINONE ANALOGS AT DAT AND SERT. F. T. Sakloth¹, J. Partilla², R. Kolanos¹, M. Barnier¹, R. Vekariya¹, P. D. Mosier¹, M. H. Baumann², & R. A. Glennon¹, ¹Department of Medicinal Chemistry, Virginia Comm. Univ., Richmond, VA 23298 & ²National Institutes of Health, National Institute of Drug Abuse, Baltimore, MD 21224. Methcathinone (MCAT) analogs are a popular class of designer drugs and, due to their abuse potential, about 15 are now controlled as US Schedule I drugs. MCAT and its simple 4-substituted analogs act primarily as dopamine and/or scrotonin releasing agents in release assays using rat synaptosomes. The goals of this project were: 1) to perform QSAR (quantitative structure-activity relationship) studies of 4-substituted MCAT analogs (n=6) to determine whether action at the dopamine and serotonin transporters (DAT and SERT, respectively) and selectivity might be explained on the basis of their steric

properties, and 2) to construct homology models of the human DAT and SERT using the crystal structure of *Drosophila melanogaster* followed by docking studies to identify how steric character might be explained on the basis of interactions with the two transporters. QSAR studies indicated that increasing length/volume of the 4-position substituent is favorable for SERT action whereas the opposite is favored for DAT. Selectivity for SERT over DAT was found to be highly correlated with volume, length and maximum width (r > 0.9). Modeling studies indicated an unfavorable interaction at hDAT, which was not seen at hSERT, in the binding pocket associated with the 4-position substituent. These differences in the binding pockets of the two transporters could explain differences in selectivity. 4-Position steric factors seemingly regulate DAT/SERT selectivity. [Supported by DA DA033930.]

QUANTIFICATION OF HUMAN OSTEOPONTIN, A CANCER BIOMARKER PROTEIN, FROM PLASMA BY MICROFLOW LC-MS/MS, M, Faria<sup>1</sup>, M. S. Halquist<sup>1</sup>, M. Yuan<sup>2</sup>, W. Mylott Jr.<sup>2</sup>, R. G. Jenkins<sup>2</sup>, & H. Thomas Karnes<sup>1</sup>, <sup>1</sup>Virginia Comm. Univ., Richmond, VA 23298, <sup>2</sup>PPD Inc, Richmond, VA 23230. Human osteopontin is a secreted plasma protein which is elevated in various cancers and is indicative of poor prognosis. It mainly operates through its integrin binding site 159 RGDSVVYGLR. A method was developed and validated for quantitative measurement of human osteopontin from plasma using microflow LC-MS/MS. In silico studies were carried out using online Protein Prospector software and Basic Local Alignment Search Tool to obtain a signature peptide. An immunoaffinity coupled LC-MS/MS method was developed. The applicability of the validated method was demonstrated by quantification of OPN from plasma samples obtained from 10 healthy individuals and 10 breast cancer patients. A biologically relevant tryptic peptide 'GDSVVYGLR' which is unique to human osteopontin was identified and used as a signature peptide for this method. The method was validated over a linear range of 25-600 ng/mL for human osteopontin. The plasma OPN concentrations in healthy individuals ranged from 38-85 ng/ml with a mean concentration of 55±15 ng/ml. A 2-12 fold increase in osteopontin concentrations, ranging from 85-637 ng/ml, were seen in breast cancer patient samples. In conclusion, microflow LC-MS/MS can be used for accurate and precise quantitative bioanalysis of proteins. This validated LC-MS/MS method can be used in clinical settings for measuring osteopontin levels from plasma.

QUINAZOLINES AS NOVEL HUMAN OCT3 INHIBITORS: STRUCTURE ACTIVITY STUDIES. Malaika D. Argade<sup>1</sup>, Xiaolei Pan<sup>2</sup>, Kavita A. Iyer<sup>1</sup>, Douglas Sweet<sup>2</sup> & Małgorzata Dukat<sup>1</sup>, <sup>1</sup>Department of Medicinal Chemistry, & <sup>2</sup>Department of Pharmaceutics, Virginia Commonwealth University, Richmond VA 23298. Globally, depression affects 20% of the population with half being refractory to current therapies. Depression is associated with low synaptic levels of the neurotransmitters scrotonin (5-HT) and/or norepinephrine (NE) regulated by high-affinity, low-capacity transporters, SERT and/or NET (uptake-1). Recently, low-affinity, high-capacity transporters, Organic Cation Transporters (OCT1-3; uptake-2) have been identified as an alternative mechanism in regulation of synaptic 5-HT and NE levels. Previously, we reported that 2-amino-6-chlorodihydroquinazoline (A6CDQ) showed antidepressant-like effects in the mouse tail suspension test. A6CDQ lacks affinity at SERT ( $K_i > 10,000$  nM), but inhibits hOCT3 (IC<sub>50</sub> = 3.9  $\mu$ M). To determine whether the 6-chloro group exerts its

effect via its electronic or lipophilic character, we synthesized the 6-methyl analog (A6MDQ) that bears an electron-donating group as opposed to the electron-withdrawing chloro group. Methyl and chloro groups possess similar lipophilic (i.e.,  $\pi=0.56;~0.71,~$  respectively), but opposite electronic character. A6MDQ inhibited hOCT3 expressed in HEK293 cells (IC $_{50}=1.6~\mu\text{M}$ ). This suggests that the lipophilic character of the substituent plays an important role in the inhibitory actions of hOCTs. We generated the first 3-D graphics model of hOCT3 using the inorganic phosphate transporter (PDB:4J05) as a template. The molecular modeling studies agreed with the experimental results obtained since the 6-methyl analog utilizes a similar binding mode as the 6-chloro analog, thereby validating the model.

QSAR AND MOLECULAR MODELING STUDIES ON ARYLGUANIDINES AS α7 nAChR NAMs. O. I. Alwassil<sup>1</sup>, S. Khatri<sup>2</sup>, M. K. Schulte<sup>2</sup> & M. Dukat<sup>1</sup>, <sup>1</sup>Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond, VA 23298, <sup>2</sup>Department of Pharmaceutical Sciences, University of Sciences, Philadelphia, PA 19104. There is ample literature evidence that α7 neuronal nicotinic acetylcholine receptors (nAChRs) might play a role in the treatment of Alzheimer's disease. Previously we reported that meta-chlorophenylguanidine (mCPG; 1) and its Nmethyl analog 2, developed in our laboratory, exert negative allosteric modulator (NAM) actions at  $\alpha$ 7 nAChRs expressed in HEK cells (IC<sub>50</sub> = 8 and 1.3  $\mu$ M, respectively). Initial SAR and 3D graphics models suggested different binding modes. Thus, two series of analogs (n = 16) based on 1 and 2 were synthesized and evaluated for functional activity in two-electrode voltage clamp assays using frog oocyteexpressed  $\alpha$ 7 nAChRs (IC<sub>50</sub> value range = 21 – 118  $\mu$ M; 12 – 125  $\mu$ M, respectively). Quantitative structure-activity relationship (QSAR) studies support our initial findings that the two series of NAMs interact in a different manner at a7 nAChRs because parallel structural changes did not result in parallel shifts of activity and there is a lack of significant correlation between the activities of the two series (r = 0.474). Furthermore, the studies indicated that electronic (?; r = 0.820) properties might be important at the *meta*-position of 1 analogs and shape (B<sub>i</sub>; r = 0.927) for the 2 series for their NAM actions, and support our proposed 3D graphics model. Supported in part by the Virginia Center on Aging (Award No. 12-2).

DECONSTRUCTION OF RISPERIDONE – HOW FAR IS TOO FAR? S. A. Gaitonde<sup>1</sup>, J. Younkin<sup>2</sup>, D. E. Logothetis<sup>2</sup>, R. A. Glennon<sup>1</sup> & M. Dukat<sup>1</sup>, <sup>1</sup>Departments of Medicinal Chemistry and <sup>2</sup>Physiology & Biophysics, VCU, Richmond, VA 23289. Ketanserin (competitive antagonist) and the antipsychotic agent risperidone (an inverse agonist), though structurally similar, differ in their functional action at 5-HT<sub>2A</sub> receptors. This study probes this disparity and attempts to envisage the minimal structural features that confer risperidone with its action. The structure of risperidone was deconstructed to give 4 analogs: 6-fluoro-3-(piperidin-4-yl)benzisoxazole (FBP), 6-fluoro-3-(1-methyl-piperidin-4-yl)benzisoxazole (FBPM), 4-[(4-(6-fluorobenzisoxazol-3-yl)piperidin-1-yl)-1-(piperidin-1-yl)]butan-1-one (FBPC) and 6-fluoro-3-[(1-(4-piperidin-1-yl)butylpiperidin-4-yl)]benzisoxazole (FBPS). The analogs were synthesized and functional action examined using two-electrode voltage clamp techniques (*Xenopus* oocytes expressing 5-HT<sub>2A</sub> receptors and Kir3.4 channels). FBPC and FBPS were partial agonists (60% and 62% activity compared to 5-HT,

respectively) whereas FBP and FBPM were competitive antagonists (74% and 100% inhibition of 5-HT activity, respectively). A 3D-graphics model of the h5-HT<sub>2A</sub> receptor using the crystal structure of the h5-HT<sub>2B</sub> receptor (PDB 4IB4) as template showed that ketanserin and risperidone differ in their binding mode at TM5 in forming hydrogen bonds with Ser239 and Ser242, respectively, and that risperidone and its analogs displayed similar binding modes and form a bidentate interaction with Ser159 (TM3); ketanserin did not. Based on the modeling results, it can be concluded that, at present, our model cannot distinguish between the observed functional actions. But, inverse agonist action of FBP/FBPM remains to be established.

ΑN ASSOCIATION BETWEEN THE DEPRESSION AND SYSTEMIC INFLAMMATION IN NHANES POPULATIONS. Rachel Smith<sup>1,2</sup>, Maria Stepanova<sup>1,2</sup>, Ancha Baranova<sup>1,2</sup>, & Aybike Birerdinc<sup>1,2</sup>, <sup>1</sup>Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church, VA 22042 and Center for the Study of Chronic Metabolic Diseases, George Mason University, Fairfax, VA 22030. A population-based study was conducted to determine the association of individuals with chronic diseases and depression from five cycles of the National Health and Nutrition Examination Survey (NHANES) data. The presence of depression was determined from a depression screener questionnaire, while the presence of chronic diseases were gathered from medical conditions questionnaires (3,751 and 26,225 subjects respectfully). Interestingly, individuals with depression and those with multimorbidity had the same statistically significant increased inflammatory markers as assessed by HOMA scores, C-reactive proteins, white blood cell counts, and percentages of segmented neutrophils and glycohemoglobin (p<0.05). Additionally, both had statistically significant decreased levels of HDL-cholesterol, lymphocyte percentages, and monocyte percentages (p<0.05). The increased inflammatory markers suggest that low-grade chronic inflammation may be the connection between depression and chronic diseases in these individuals. Examining these inflammatory markers may be used as predictors in the treatment of patients.

MITOCHONDRIAL HAPLOGROUP ASSESSMENT IN OBESE PATIENTS WITH NAFLD. Kianoush Jeiran<sup>1</sup>, Rohini Mehta<sup>2</sup>, Ancha V, Baranova<sup>1</sup>, & Zobair Younossi<sup>2</sup>, School of Systems Biology, George Mason University, Manassas, VA 20110 and <sup>2</sup> Claude Moore Health Education and Research Center, Inova Fairfax Medical Campus, Falls Church, VA 22042. Liver disease is considered a significant health problem worldwide nowadays. Among distinct disease, non-alcoholic fatty liver disease (NAFLD), which is widely considered the hepatic manifestation of the metabolic syndrome, is a complex multifactorial disease trait where environment and genetic variations interact to determine the wide spectrum of disease progression. One of the key challenges is to predict the progression of NAFLD; mitochondrial genotyping may serve as an indicator of increased susceptibility to progressive NAFLD. In this study the association of sequence variations (haplogroups and indels) in control loop of mitochondria within 86 patients with biopsy-proven NAFLD and metabolic syndrome was investigated. After extracting DNA from whole blood cells and amplifying control loops by PCR and sequencing the control region; samples have been categorized within 11 haplogroups (H, I, J, K, L, M, T, U, V, W, and X). Liver biopsy pathology has revealed that 64 (74.4%) patients diagnosed with NAFLD and 22 (25.6%) patients had

normal biopsy. Moreover, 80.8% of patients with Non-L mtDNA haplogroups progress to NAFLD, but only 38.8% of patients with L haplogroup diagnosed with NAFLD which proves that obese patients with L haplogroup are much less susceptible to develop non-alcoholic fatty liver comparing to other mtDNA genotypes.

### Posters

REGULATION OF SEROTONIN TRANSPORT FUNCTION AND CELL SURFACE EXPRESSION BY BRAIN-DERIVED NEUROTROPHIC FACTOR. Rajamanickam, K. N. Najdu, L. Jayanthi, & S. Ramamoorthy, Dept. of Pharm, & Tox., VCU, Riehmond VA 23298. The scrotonin (5-HT) transporter (SERT) maintains the level of serotonin in the synaptic cleft by reuptaking released 5-HT into presynaptic terminals. Dysfunction of SERT leads to several psychiatric disorders like autism, depression, obsessive-compulsive disorder, and addiction, Selective serotonin reuptake inhibitors (SSRIs) are specifically target SERT and inhibit 5-HT reuptake in the treatment of psychiatric disorders. A member of neurotrophin family of proteins, brain derived neurotrophic factor (BDNF), which binds to Tropomyosine-related kinase B receptor (TrkB), reduces SSRI mediated inhibition of 5-HT reuptake by increasing SERT activity. Current study was aimed to understand the regulation of activity and expression of SERT by BDNF and TrkB. BDNF treatment of HEK 293 cells coexpressing SERT and TrkB increased the SERT activity parallel with increased surface level of SERT protein. Kinetic assay revealed increase in Vmax of SERT following BDNF treatment, BDNF treatment also increased the phosphorylation of endogenous protein kinascs ERK1/2 and Akt in the HEK 293 cells, Treatment of HEK 293 cells with the inhibitors of ERK1/2 (PD98059) and Akt (Akt inhibitor X - AktX) reduced the 5-HT uptake in a concentration dependent manner. BDNF treatment reversed the inhibitory effect of PD98059 but not of AktX on SERT activity. This result indicates that BDNF may stimulate the 5-HT uptake indirectly through increasing the phosphorylation of ERK1/2. The overall observations suggest that BDNF regulates surface expression and function of the scrotonin transporters.

COMPARISON OF THE COGNITIVE EFFECTS OF THE NMDA ANTAGONIST KETAMINE IN ADOLESCENT AND ADULT RATS. M. P. George and K. L. Nicholson, Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA 23298. Ketamine has been subject to growing rates of abuse worldwide, particularly in adolescent populations. Previous studies in the laboratory have shown differences in abuse-related behavioral effects of ketamine in rats based on age. The current project extends this investigation by comparing the effects of ketamine on memory in a novel object recognition procedure in male Sprague-Dawley rats. Activity in a 17 in. x17 in. experimental chamber was recorded and analyzed using ANYmaze software, Rats underwent two 6-min training periods, separated by I hour, during which two like objects were present in the chamber. After an hour, one object was replaced with a novel item and rats returned to the chamber for a 6-min testing period. Time spent in the two zones when the like items were present was compared to the times when one item was replaced with a novel object. Baseline performance was determined across three age groups: adolescent (PND34-44), young adult (PND70-90) and older adult (PND150-180). Subsequently ketamine and cocaine

were tested in adult rats for disruption of memory retrieval. In baseline tests, adolescents spent more time in the novel object zone, however due to a high level of variability, the effect was not significant. Both young and older adult rats demonstrated evidence of learning with significantly more time spent with the novel object. Cocaine and ketamine produced dose-dependent disruption of memory retrieval in both groups of adult rats. The effect of additional habituation and training in adolescent subjects is being investigated prior to drug testing in this age group.

DEVELOPMENT OF NATURAL CYCLIC PEPTIDE INHIBITORS OF XRCC4/XLF INTERACTION FOR RADIO-SENSITIZATION OF BREAST TUMOR CELLS. M. Al Mohaini<sup>1</sup>, K. Akopiants<sup>1</sup>, E. R. White<sup>2</sup>, M. C. T. Hartman<sup>2,3</sup>, & L. F. Povirk<sup>1,3</sup>, Dept. of Pharm. & Tox., <sup>2</sup>Dept, of Chemistry & <sup>3</sup>Massey Cancer Center, VCU, Richmond, VA 23298. Breast cancer is the second leading cause of cancer death in women, and the standard treatment is ionizing radiation combined with surgery and chemotherapy. Cell death after exposure to radiation results largely from DNA double-strand breaks. Previous studies showed that many breast tumor cells depend mainly on nonhomologous end joining (NHEJ) for repairing induced DNA damage. XRCC4 and XLF are two essential interacting proteins in the NHEJ process. A single mutation on the XLF-binding interface of XRCC4 at M61, F106, E55 or D58 has been shown to disrupt its interaction with XLF, thus inhibiting NHEJ. Therefore, it is proposed that small natural cyclic peptides that bind to the XLF interface of XRCC4 near M61 and F106 can be identified through an mRNA display in vitro selection, and these peptides will inhibit NHEJ and thereby radiosensitize breast tumor cells. Using mRNA display, we created a library of a trillion unique cyclic mRNA-peptide fusions. After seven rounds of in vitro selection, the eluted fusions were cloned and sequenced. Results showed homology of sequences of five main families (Pep 7.1-7.5), and we have synthesized representative peptides of these families. Fluorescence polarization studies showed binding affinities of Pep 7.1 and Pep 7.2 to XRCC4<sup>157</sup> protein with K<sub>p</sub> of 16.78 and 18.77, respectively. Furthermore, Pep 7.1 and Pep 7.2 were able to inhibit the rejoining of a simple restriction-cut plasmid in an extract of patient derived XLF Bus cells supplemented with XLF protein,

THE ROLE OF P38 NITORGEN-ACTIVATED PROTEIN KINASE MEDIATED NOREPINEPHRINE TRANSPOTER REGULATION IN COCAINE-INDUCED BEHAVIOR. P. Mannangatti, K. Narasimha Naidu, M. I. Damaj, S. Ramamoorthy, & L. D. Jayanthi, Dept. of Pharm & Tox., VCU, Richmond, VA 23298. The noradrenergic and p38 mitogen activated protein kinase (p38 MAPK) systems have been implicated in behavioural effects of cocaine. The present experiments were designed to probe functional interactions between norepinephrine transporter NET and p38 MAPK by testing the role of p38 MAPK-mediated NET regulation in cocaine-mediated behaviours. In Experiment 1, mice pre-exposed to i.p. cocaine (15 mg·kg-1, day 1; 30 mg·kg-1 days 2-4; and no drug, days 5-8) or saline were pretreated with SB203580 (50-100 μg·kg-1, i.p), before testing for locomotor sensitization in response to cocaine challenge (15 mg·kg-1, day 9). In Experiment 2, mice were subjected to unbiased cocaine CPP where mice received 10 mg·kg-1, i.p cocaine for 4 days and on day five received vehicle or SB2035804-[5-(4-Fluorophenyl)-2-[4-(methylsulfonyl)phenyl]-1H-imidazol-4-I]pyridine (50-100 μg·kg-1, i.p), and tested for

preference scores. In Experiment 3, after behavioural testing, NE transport function and NET expression levels along with phospho-p38 MAPK levels were measured in the prefrontal cortex (PFC) and nucleus accumbens (NAc) of the mice. Pretreatment with SB203580 selectively interfered with cocaine-induced locomotor sensitization and CPP. In addition, SB203580 pretreatment blocked cocaine-induced p38 MAPK activation and the upregulation of NE transport and NET expression in the PFC and NAc brain regions of the mice. These findings reveal a mediating role for p38 MAPK-mediated NE transmission in cocaine-related behaviours.

THE DUAL FAAH/MAGL INHIBITOR SA-57 SERVES AS A DISCRIMINATIVE STIMULUS. R. Owens', B. Cravatt<sup>2</sup>, & A. Lichtman<sup>1</sup>, <sup>1</sup>Dept, of Pharm, & Tox., VCU, Richmond, VA 23298, <sup>2</sup>The Skaggs Institute, & TSRI, La Jolla, CA 92037. The drug discrimination paradigm is a very sensitive assay to infer interoceptive effects of a variety of psychoactive drugs, including cannabinoids. The phytocannabinoid  $\Delta^9$ tetrahydrocannabinol (THC) serves as a discriminative stimulus in rodents via cannabinoid receptor type one (CB<sub>1</sub>) mechanism of action. Simultaneous inhibition of the endocannabinoid degradative enzyme fatty acid amide hydrolasc (FAAH) and monoacylglycerol lipasc (MAGL) produces THC-like effects in this paradigm. More recently, the development of the dual FAAH/MAGL inhibitor SA-57, which is more potent at inhibiting FAAH than MAGL, provides a useful tool to investigate the impact of differentially inhibiting these endocannabinoid degradative enzymes. Mice were trained to discriminate between the potent synthetic cannabinoid CP55,940 (0.1 mg/kg) and vehicle in a nose-poke discrimination task (ED<sub>50</sub>(95% confidence interval) = 0.34 0.058 - 1.99) mg/kg, SA-57 (10 mg/kg) fully substituted for CP55,940. The discriminative stimulus effects of both CP55,940 and SA-57 were completely antagonized by 3 mg/kg rimonabant, indicating a CB, receptor mechanism of action. These findings suggest that SA-57 produces a similar interoceptive stimulus as CP55,940. Importantly, mice learned to discriminate between SA-57 and vehicle in a dose-related fashion (ED<sub>50</sub> value = 4.49 mg/kg, 95% Cl 3.77 - 5.35 mg/kg). This study represents the first demonstration that complete blockade of the primary endocannabinoid degradative enzymes FAAH and MAGL serves as a reliable discriminative stimulus.

ADIPONECTIN EXPRESSION IN PATIENTS SUFFERING FROM METABOLIC DISORDERS. Kameron M. Tavakolian & Michael Estep, The Beatty Liver & Obesity Research Program, Inova, Falls Church, VA 22042, School of Systems Biology, College of Science, George Mason University, Fairfax, VA 22030. Adiponectin is an essential adipokine released from adipose tissue with antiatherogenic, anti-diabetic, and anti-inflammatory propertics. Individuals with fatty liver discase show reduced circulating levels of adiponectin independent of body mass index, which is inversely correlated to the degree of steatosis and inflammation. The aim of this study was to measure the expression of adiponectin and its receptor, AdipoR1, in visceral adipose tissue and its relation to hepatic fibrosis. RNA was extracted from visceral adipose tissue from a selected cohort of 19 morbidly obese patients using a BIORAD kit. The iScript cDNA synthesis kit was used and RT-PCR was performed with AdipoQ and AdipoR1 primers, along with GAPDH and RP2 as endogenous controls. Primers for the study were designed on NCBI with stringent criteria such that each primer crossed

an intron and the deltaG setting minimized hairpin formation and primer dimerization. No correlation between adiponectin expression and severity of fibrosis was observed, although a larger sample size would be needed to determine whether adiponectin plays a role in fibrosis.

HYDROXYMETHYLATION AND ALZHEIMER'S DISEASE: A MAP OF DYNAMIC REGIONS IN THE DISEASED STATE. R. H. Haraf, M. Baker, J. Davy, A. Hazy, D. Harrison, N. Taher, and G. Isaacs, Dept. of Biology, Liberty University, Lynchburg VA 24502. Alzheimer's Disease (AD) is a neurodegenerative disorder that is currently responsible for approximately 500,000 deaths per year. Because less than 5% of cases result from a genetic mutation, epigenetic factors are being considered as a potential cause for the majority of AD patients. Hydroxymethylation, a recently discovered epigenetic modification to cytosine, has been found to be especially prevalent and active in the brain. Furthermore, it has been linked to neurogenesis, neural differentiation, and normal neurological function. For this reason, understanding the exact role of hydroxymethylation in the brain may shed light on the cause and pathogenesis of AD, as well as other neurological disorders. This study sought to map the distribution of hydroxymethylation in the brain of a healthy mouse and a mouse displaying AD-like characteristics. Using a double transgenic murine model that developed beta-Amyloid plaques in the brain, hippocampal DNA was tested for the presence of hydroxymethylation in the promoter regions of genes. Changes in hydroxymethylation distribution between the healthy and AD mice were mapped, allowing the determination of 206 specific genes of interest that may be dysregulated in Alzheimer's Disease.

THE EFFECTS OF DOXYCYCLINE-INDUCED HIV-1 TAT **PROTEIN** EXPRESSION IN MICE ON THE STARTLE REFLEX AND ITS PREPULSE INHIBITION. R. M. Enga, K. F. Hauser, & P. M. Beardsley, Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA 23298. Motor and behavioral deficits, including deficits of attention, are present in HIV-associated neurocognitive disorders (HAND) that may, in part, be attributable to HIV-1 Tat protein expression. Sensorimotor gating is linked to attention, and can be measured by the acoustic startle response and its prepulse inhibition (PPI). To determine whether HIV Tat contributes to impaired sensorimotor gating, we tested doxycycline (DOX)-inducible Tat transgenic mice and C57BL/6J (background strain) mice for baseline PPI to a 119 dB startle pulse with 73, 77, and 85 dB prepulses. The mice were re-evaluated 2, 9, and 16-d post-DOX administration. Locomotor activity was also assessed in a 2-h test session in naïve Tat transgenic mice. Baseline PPI was not significantly different between groups; however, PPI significantly decreased in Tat(+)/DOX after 2, 9, and 16-d of DOX administration (p  $\leq$  0.05). PPI was unaltered in DOX-treated Tat(-) and C57BL/6J mice, indicating a lack of nonspecific DOX effects. PPI was also unaltered in untreated Tat(+), Tat(-), and C57BL/6J mice. In locomotor activity tests, baseline total distance traveled was not significantly different between groups, however after DOX administration, Tat(+)/DOX mice traveled significantly less than Tat(-)/DOX micc (p≤0.05). These results suggest that HIV Tat induction caused sensorimotor gating and locomotor deficits in mice, which may serve

as predictive correlates of some aspects of the behavioral and motor pathology associated with HAND.

MITOCHONDRIAL DYSFUNCTION IN STREPTOZOTOCIN ALZHEIMER'S DISEASE MODEL. Essie S. Komla<sup>1</sup>, Ann C. Rice<sup>1</sup>, Paula I. Moreira<sup>2</sup>, & James P. Bennett Jr., 'Parkinson's and Movement Disorders Center, Virginia Commonwealth University, Richmond, VA 23298 & <sup>2</sup>Center for Neuroscience and Cell Biology of Coimbra, University of Coimbra, 3030 Coimbra, Portugal. Alzheimer's disease (AD) is the most common type of dementia found in the adult population. The disease is characterized by death or malfunction of neurons leading to memory loss, cognitive decline, and increased level of amyloid beta peptides and neurofibrillary tangles. Some researchers suggest AD is an "insulin-resistant brain state" disorder and the use of intracerebroventricular (icv) injection of streptozotocin (STZ) in rats is emerging as an experimental model for the disease. We hypothesize we will observe increased expression of mitochondrial biogenesis markers in the icvSTZ rats as a compensatory response to decreased metabolism. Using laser capture microdissection (LCM) to collect multiple groups of 20 hippocampal pyramidal neurons, we determined the mitochondrial DNA (mtDNA) copy numbers from 5 ievSTZ rats and 4 control (CTL) cases using quantitative polymerase chain reaction (qPCR). We found no significant difference between the mtDNA copy numbers of the STZ samples compared to the control. However, these preliminary data show a trend toward increased mtDNA copy numbers in STZ treated brain which is one of the biomarkers of mitochondrial biogenesis. In conclusion, our preliminary data show an increase in mitochondrial gene copy numbers as the cells try to compensate for the degeneration of the neurons; thereby, increasing mitochondrial synthesis.

THERAPEUTIC EFFICACY OF COMBINATION OF MTOR INHIBITORS AND AMPK ACTIVATORS IN NON-SMALL CELL LUNG CANCER. Grinal M. Corriea & Richard G. Moran, Dept. of Pharmacology & Toxicology, Richmond, VA 23298. Pemetrexed (PTX), an antifolate drug, has been approved by the FDA for first line therapy of mesothelioma and non-small cell lung cancer. In addition to its primary site of action on thymidylate synthase, PTX also inhibits the second folate-dependent enzyme of purine biosynthesis, aminoimidazolecarboxamide ribonucleotide formyltransferase (AICART). Accumulation of AICART substrate, ZMP in PTXinhibited cancer cells leads to downstream activation of AMP-activated protein kinase (AMPK) with subsequent inhibition of mammalian target of rapamycin (mTOR) and hyperphosphorylation of its downstream targets responsible for protein synthesis and cell proliferation. Inhibitors of mTORC1 like Rapamycin and its analogs have only partial effect, as they do not inhibit mTORC2, which phosphorylates Akt subsequently relieving the inhibition of mTORC1 and leading to poor cytotoxicity. AZD8055 is an ATP-competitive inhibitor of mTOR kinase and potently inhibits both mTORC1 and mTORC2. Our data shows that AMPK activation via PTX-mediated AICART inhibition in combination with direct mTOR inhibition by AZD8055 has a synergistic interaction on the proliferation of cells in culture, Activators of AMPK enhance the effects of direct inhibitors of mTOR and vice-versa and these effects are mediated via combined effects on mTORC1. Taken together, it is a promising therapeutic strategy.

SENSITIZATION OF NON-SMALL CELL LUNG CANCER (NSCLC) TO RADIATION BY VITAMIN D BY A CYTOSTATIC FORM OF AUTOPHAGY. K. Sharma, R. W. Goehe, X. Di, S. Torti, F. Torti & D. A. Gewirtz, Dept. of Pharm. & Tox., VCU, Richmond, VA 23298. The standard of care for unresectable lung cancer is chemoradiation. However, therapeutic options are limited and patients are rarely cured. In A549 and H460 non-small cell lung cancer (NSCLC) cells, 1,25-D, (the hormonally active form of vitamin D) and EB 1089 prolonged the growth arrest induced by radiation alone and suppressed proliferative recovery, which translated to a significant reduction in clonogenic survival. In H838 or H358 NSCLC cells, which lack the vitamin D receptor or functional p53, respectively, 1,25-D, failed to modify the extent of radiation-induced growth arrest. Sensitization to radiation in H1299 NSCLC cells was evident only when p53 was induced in otherwise p53 null H1299 NSCLC cells. Sensitization was not associated with increased DNA damage or an increase in apoptosis, necrosis or senescence. Instead sensitization appeared to be a consequence of the conversion of the cytoprotective autophagy induced by radiation alone to a novel cytostatic form of autophagy by the combination of vitamin D with radiation. While both pharmacological and genetic suppression of autophagy or inhibition of AMPK phosphorylation sensitized the NSCLC cells to radiation alone, inhibition of the cytostatic autophagy induced by the combination treatment reversed sensitization. Taken together, these studies have identified a unique cytostatic function of autophagy that appears to be mediated by the vitamin D receptor, p53 and AMPK in the promotion of an enhanced response to radiation by 1,25-D, and EB 1089 in NSCLC.

CHRONIC MORPHINE ENHANCES NICOTINE RESPONSES IN SINGLE MOUSE ENTERIC NEURONS. Aravind R. Gade, William L. Dewey and Hamid I. Akbarali, Deptartment of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA 23298. Opioids are excellent pain relievers. A major side-effect of chronic opioid treatment is constipation whereas withdrawal following chronic exposure leads to diarrhea and increased gastrointestinal motility. These effects of chronic opioids are mediated by mu opioid receptors expressed on enteric neurons. Previous in-vitro studies have shown that chronic opioids enhance sensitivity to nicotinic receptor agonists. In this study we examined the sensitivity to nicotine following short (10 minutes) and long-term exposure (16-20 hours) to morphine in isolated mouse single enteric neurons using patch-clamp techniques. Nicotine (1mM) induced inward currents from holding potential of -60 mV that were significantly increased in neurons exposed for long-term but not short-term to morphine. However a significant shift was not seen in the EC (50) values in all neurons. The maximal current densities induced by 1mM nicotine were  $168 \pm 29 \text{ pA/pF}$ ,  $135 \pm 31 \text{ and } 296 \pm 100 \text{ m}$ 51 pA/pF in control, short-term and long-term morphine treated cells respectively, and the EC (50) values were  $44 \pm 17 \, \mu M$  (n = 9),  $42 \pm 14$  (n = 5) and  $26 \pm 7 \, \mu M$  (n = 11) in the same order, Shift in the maximal currents but not in the EC (50) values suggests a shift in the efficacy but not potency of nicotine after long term exposure to morphine. Studying the mechanisms involved in this enhancement is hypothesized to give a better insight into methods for treating opioid dependent and withdrawal symptoms.

# Natural History and Biodiversity

USING CAMERA TRAPPING TO MONITOR WILDLIFE IN STAFFORD COUNTY, VIRGINIA. Bryan Finch & Werner Wieland, Dept. of Biological Sciences, University of Mary Washington, Fredericksburg, VA. We employed cameras (Spy Point Model IR-5) from June 2013 to April 2014 on a 260 acre partial in Stafford County about 7.5 miles northwest of Fredericksburg, Eighteen cameras were placed in a series of 500 m<sup>2</sup> quadrants, (one per quadrant) throughout the property. Photographs were examined to identify species, calculate relative abundance (overall and seasonal), and determine activity patterns, Seasons were designated as Summer (Jun., Jul., Aug.), Fall (Sept., Oct., Nov.), Winter (Dec., Jan., Feb.), and Spring (Mar., Apr., May). A total of 3,441 trap days (1 trap day = 18 cameras x # of days - malfunctions) yielded 1,427 photographs of identifiable animals out of a total of 24,357 photographs taken. Fifteen species of birds and mammals were detected including black bear, white-tail deer, coyotcs, red and gray fox, and wild turkeys. The overall relative abundance index (RAI) for white-tail deer (9.65) and gray squirrel (3.14) were the highest, followed by wild turkey (1.74), racoon (1.67) and fox (1.67; red and gray combined). All other species observed had an RAI below 1.0. White-tailed deer had the greatest abundance in all seasons (Su = 12.29, F = 12.38, W = 7.22, Sp = 4.41), however, the RAI varied seasonally for other species. The species with the second highest RAI in the Summer and Fall was the grey squirrel (SU = 8.80, F = 3.42) whereas the wild turkey and racoon were the second most abundant in the Winter (1.74 each). Fox were the second most abundant species in the Spring (2.94). Black bear were photographed in the Summer (0.66) and Fall (0.09) only. All photographs included information on ambient temperature and this information is currently being reviewed. This project was supported by the UMW Summer Science Institute.

SALAMANDER MIGRATION AND POPULATION MONITORING WARRENTON, VA. Melissa A. Fuerst & Dr. Thomas C. Wood, New Century College, George Mason Univ., Fairfax, VA.22030-4444. The Southeastern United States has the greatest diversity of salamanders in the world with over 75 different species, and the wetland and forest habitats of these creatures have been in decline with increased pollution and habitat destruction. The purpose of this project is to monitor a population of Jefferson (Ambystoma jeffersonianum) and Spotted (Ambystoma maculatum) Salamanders in a two-year-old constructed ridge-top vernal pool at Environmental Studies on the Piedmont in Warrenton, VA as part of a long term strategy to increase breeding capacity of mole salamanders. Monitoring began in May of 2013 and is currently on-going. In the first year the pool was inhabited by Jefferson and Spotted Salamanders, Wood Frogs, Pickerel Frogs, Green Frogs, Grey Tree Frogs, and Red-Spotted Newts. An estimated 1,300 Wood Frogs and mole salamanders used the pool in its first year, a remarkable number for a newly constructed pool. Because amphibian populations are generally declining, we intend to study this successful population by measuring the growth of each new generation for the next three years. We will correlate growth measurements of the salamanders with soil temperature, water temperature, air temperature, and rainfall data. In this presentation we will summarize use patterns by amphibians for the first two years of this vernal pool; in early May of 2013, the average size of a larval salamander was 36 mm and in mid-August 2013 the average size was 61 mm.

COMPARISON OF SPECIES-SPECIFIC COURTSHIP SONGS WITHIN A GENUS OF PARASITIC WASPS. Justin P. Bredlau & Karen M. Kester, Integrative Life Sciences & Department of Biology, Virginia Commonwealth University, Richmond VA 23284. In many animals, male courtship songs provide a signal that females use to identify conspecifies and assess mate quality, and thus play an important role in premating isolation based on sexual selection. We compared male courtship songs among species of Cotesia (Hymenoptera: Braconidae), which is perhaps the most speciose genus of parasitic wasps. Prior research on four Cotesia spp. has demonstrated that songs are generated by wing fanning and pulses in stereotypical patterns. We eharaeterized courtship songs produced by an additional eight of ~80 described species of Cotesia that occur in North America, Songs of cmergent males from wild caterpillar hosts were recorded with miniature omnidirectional microphones in a noise reduction booth. Pattern, frequency, and duration of song components were analyzed using Raven Pro. Species-specific songs varied significantly in structure and duration of repeating pulse and buzz components, and in fundamental frequency (175 to 328 Hz). Differences in courtship songs loosely mirrored the proposed molecular phylogeny by Michel-Salzat & Whitfield (2004) in that songs of more closely related species were more similar than those of more distantly related species. Courtship song analysis may aid in identifying closely related cryptic species and provide insight into the evolution of this highly diverse and agriculturally important taxon.

WHICH SPECIES OF ANISOPTERA WILL FIRST COLONIZE OR RECOLONIZE A LENTIC HABITAT. Richard S. Groover, Dept. of Environmental Science & Policy, George Mason Univ., Fairfax VA 22030-4444. A six year study of lentic sites in Hanover County, VA, was conducted. First, in a county-wide survey, 26 sites were sampled to identify available dragonflies for colonization. Seven new species not previously reported in Hanover County were found. Nine new or reconstructed impoundments were studied to identify which species first colonized these sites. Predictions may be made based on the data from this research.

LEAST-COST PATH ANALYSIS OF MOVEMENT BY RACCOONS ON THE VIRGINIA BARRIER ISLANDS. R. D. Dueser<sup>1</sup>, J. H. Porter<sup>2</sup> & N. D. Moncrief<sup>3</sup>, Dept. Wildland Resources, Utah State University, Logan, UT 84322, Dept. Envi. Sci., UVA, Charlottesville, VA 22904, & 3VA Museum of Natural History, Martinsville, VA 24112. Predation by raccoons (*Procyon lotor*) has been implicated in the recent decline of beach-nesting and colonial waterbirds, including species that are endangered and threatened, on the Virginia barrier islands. Raccoons are established on 11 islands and occur sporadically on others. We seek methods to control raccoon access to particular islands during the bird nesting season. We applied least-cost path analysis to quantify potential movements of raccoons in this landscape in order to 1) assess the relative roles of mainland and island populations as sources of immigrants, and 2) identify potential transit pathways for focusing monitoring and control efforts. We estimated movement costs across upland, marsh and open-water habitats. We conclude that inter-island movements are typically less costly for raccoons than immigration

from the mainland, and we identify islands where predator control is most likely to be useful in enhancing avian recruitment. A similar approach can be applied to any landscape where there are distinct differences in the costs of traversing different elements of the landscape.

THE EFFECTS OF URBANIZATION ON THE RELATIONSHIP AMONG BIRDS, TICKS, AND TICK-BORNE PATHOGENS. E. L. Heller, E. L. Walters, & H. D. Gaff, Department of Biological Sciences, Old Dominion University, Norfolk VA 23529. The Hampton Roads region of Virginia is one of the largest urban areas within the Atlantic Flyway, one of four major avian migratory flyways in North America. At least 500 species of birds, 40% of which are of conservation concern, use this flyway; thus, understanding factors affecting species mortality is of paramount concern. By capturing and banding birds at sites of varying levels of urbanization within the Hampton Roads urban matrix, we study the roles birds play in tick-borne pathogen transfer. We set-up mistnets at 5 permanent sites starting July 2012 in order to catch, band, draw blood, and collect ticks from migratory and resident birds. The ticks are identified to species in order to determine what tick species have preferred avian hosts. Both the ticks and avian blood are tested for zoonotic diseases. Results show that the proportion of birds caught with ticks is greater at less urbanized sites than at more urbanized ones and that ticks are more commonly found on ground-foraging species such as Thryothorus ludovicianus. The most common ticks collected from birds thus far are larval Haemaphysalis leporispalustris. Several ticks collected off birds have tested positive for Borrelia burgdorferi. Seasonal trends show that mid-September to carly November are the most productive for catching birds, likely due to fall migrations. This study demonstrates how levels of urbanization can influence avian host choice by ticks and increases knowledge of the corresponding relationship between urbanization and disease pathogen prevalence. This study was funded in part by the Virginia Academy of Science and Old Dominion University.

RECONSTRUCTING PATTERNS OF MICROENDEMISM IN MADAGASCAN SEASONALLY DRY TROPICAL FORESTS: DATING THE DIVERGENCE OF FOUR RADIATIONS IN THE MYRRH GENUS, COMMIPHORA JACQ. (BURSERACEAE) USING A FIVE-MARKER PHYLOGENY. M. R. Gostel & A. Weeks, George Mason University, Dept. of Environmental Science and Policy, Fairfax, VA 22030-4444. The myrrh genus, Commiphora Jacq., is the most species rich in the Burseraceae, yet its diversity is poorly understood. We have sampled approximately 61% of the species in Commiphora and over 90% of the species from Madagascar. A nearly comprehensive sampling of Madagascan Commiphora has contributed to a molecular phylogeny based on two nuclear (ETS and ITS) and three chloroplast spacers (ndhF-rpl32, psbA-trnH, and trnD-trnT). Previous studies have suggested that Commiphora has resulted from two dispersal events from continental Africa to Madagascar; however, we report a minimum of four such dispersal events. We have estimated the timing of these dispersal events and propose areas of endemism using species range and geographic information. Using this information, we perform ancestral area reconstruction to test three biogeographic hypotheses for each of the four radiations of Madagascan Commiphora. We identify shortcomings for understanding diversification in Madagascar and suggest mechanisms to overcome such challenges.

REOUIREMENTS OF RARE PLANTS ECOLOGICAL AND MANAGEMENT IMPLICATIONS FOR THE ABRAMS CREEK WETLANDS IN WINCHESTER AND FREDERICK COUNTY, VIRGINIA. Ashley L. Landes & Woodward S. Bousquet, Environmental Studies Program, Shenandoah University, Winchester, VA 22601. The Abrams Creek Wetlands (ACW) are home to over 20 of Virginia's rare plant species and at least two ecological communities classified as rare on a statewide basis. They were first examined by the Virginia Natural Heritage Program (VNHP) in 1980. Since 1997, Shenandoah University faculty and students in the Environmental Studies Program have been investigating this site in collaboration with the VNHP, Current research is part of a three-year floristic study. The purposes were to compile information from existing literature on the ecological requirements and biogeography of the rare species, prepare a one-page species profile for each, and develop recommendations for managing the Abrams Creek Wellands to protect its rare flora and ceological communities. During the limited time available, species profiles of four rare plant species in the Abrams Creek Wetlands were compiled. Each presents an analysis of ecological requirements and distribution patterns. Cold springs and calcium-rich soils allow several of the site's rare species to flourish as disjunct populations. Review of the 2007 Abrams Creek Wetlands Preserve Management Plan identified the need to accelerate plans for selective removal of invasive native trees, and the maintenance or implementation of light grazing regimes in several locations.

### **Posters**

SMALL MAMMALS FROM AN ISOLATED, REMNANT CLOUD FOREST IN GUATEMALA. R. P. Eckerlin<sup>1</sup>, J. O. Matson<sup>2</sup>, N. Ordóñez Garza<sup>3</sup>, W. Bulmer<sup>1</sup>, & S. Greiman<sup>4</sup>, <sup>1</sup>Natural Sciences Division, Northern VA Comm. Coll., Annandale, VA 22003, Department of Biological Sciences, San Jose State Univ., San Jose CA, 95192, <sup>3</sup>Department of Biological Sciences, Texas Tech Univ., Lubbock, TX 79409, & <sup>4</sup>Department of Biology., Univ. North Dakota, Grand Forks, ND 58202. As part of a long term effort to determine the community structure of small mammals inhabiting highland habitats in Guatemala we surveyed a remnant isolated mixed hardwood cloud forest (2640m elevation) at Finca El Pilar, 11km SE La Antigua, Sacatepequez, Guatemala, Removal trapping using a combination of live traps and snap traps and pitfalls for 5 nights (4-9 January 2013) for 968 trap nights and 620 pitfall nights resulted in 46 captures of 10 species of marsupials, shrews, and rodents. This diversity and having one dominant species, Peromyscus guatemalensis (n=20, 44%) is similar to other Central American cloud forest sites. Other less abundant species were Cryptotis goodwini (n=6), Sorex veraepacis (n=5), Reithrodontomys sumichrasti (n=4), Handleyomys rhabdops (n=3), Heteromys desmarestianus (n=2), Peromyscus beatae (n=2), Reithrodontomys mexicanus (n=2), Marmosa mexicana (n=1), and Nyctomys sumichrasti (n=1). We evaluated the reproductive condition of these small mammals and collected internal and external parasites. Although the habitat in this cloud forest is somewhat disturbed, the species diversity for this small mammal community remains relatively high as indicated by the presence of 10 species.

FUNCTIONAL GENOMICS OF DEEP SEA MICROBIOMES. Christine A. McGown & Leila J. Hamdan, Microbiome Analysis Center, George Mason Univ.,

Manassas, VA 20110. Deep-sea microbiomes are dominated by unidentified phylotypes with uncharacterized functional roles. Bioinformatics pipelines can predict these functional genetic pathways from environmentally stable DNA sequences. For this study sediment samples from three locations across the Alaska Beaufort Shelf were utilized. The 16S-rRNA genes of the samples were analyzed with multitag pyroseuqueing during a previous investigation. Operational Taxonomic Units were selected using the Quantitative Insights Into Microbial Ecology (QIIME) pipeline. Subsequently taxonomical classification and beta-significance indices were derived. Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) analysis was performed to extract metabolic pathways in the sample set. Results indicate that sulfur metabolism genes were only slightly enriched (0.268%-0.424% abundance) in the samples. Spearman Rank Correlation analysis was used to determine associations between derived metabolic function from PICRUSt and measured abiotic parameters and metabolic rates, Despite relatively low abundance, sulfur metabolism genes correlated significantly with measured sulfate reduction rates at one location where (p = 0.492). This study demonstrates the value of incorporating functional genomics in environmental microbiology studies, as it establishes links between environmental factors and the unresolved genetics of an ecosystem.

STATUS OF COMMON WINTERING BIRDS IN THE CENTRAL PIEDMONT OF VIRGINIA. Caryn D. Ross, Sujan M. Henkanaththegedara, & Mark L. Fink, Department of Biological & Environmental Sciences, Longwood University, Farmville VA 23909. Several studies have shown a steady decline of common breeding birds in the eastern United States including Virginia, However, the long-term population dynamics of common wintering birds are poorly understood, especially for the Piedmont region of Virginia. We studied the long-term population dynamics of 12 common wintering birds in central Piedmont using Christmas Bird Count data from five count circles (Darlington Heights, Lynchburg, Warren, Gordonsville & Lake Anna). Linear regression models revealed significant population declines (p < 0.01) for Northern Cardinal (Cardinalis cardinalis), Carolina Chickadee (Poecile carolinensis) and Mourning Dove (Zenaida macroura), and significant population increases (p < 0.0001) for Eastern Bluebird (Sialia sialis), Turkey Vulture (Cathartes aura) and Redtailed Hawk (Buteo jamaicensis). Additional analysis comparing average bird densities between Pre- and Post-1980 suggested significant declines (p < 0.05) of Northern Cardinal (C. cardinalis), Carolina Chickadee (P. carolinensis), Tufted Titmouse (Baeolophus bicolor) and Mourning Dove (Z. macroura). Future research involves expanding the analysis to additional species and studying correlations between population trends and climate variables.

INTER-POPULATION MORPHOLOGICAL VARIATIONS IN ENDANGERED MOHAVE TUI CHUB (SIPHATELES BICOLOR MOHAVENSIS). <u>Brady P. Donovan, Samuel P. Hull</u>, & Sujan M. Henkanaththegedara, Department of Biological & Environmental Sciences, Longwood University, Farmville VA. 23909. The phenotype of an organism is influenced by the habitat it lives in. Translocated populations of threatened species may show rapid divergence of phenotype (e.g. body shape) compared to the source population due to habitat mismatches. Such is the case with endangered Mohave tui chub (*Siphateles bicolor mohavensis*), a desert fish

species restricted to the Mojave River, California. The historical river populations were extirpated by late 1960s and currently there are 6 Mohave tui chub populations as a result of translocation efforts. We studied body shape of tui chubs collected from the parental population and two other translocated populations in man-made habitats. We established 9 body shape landmarks and measured fish (N = 85) for 13 morphometric measurements. We compared body shapes using a series of One-way ANOVAs run on 13 morphometric measurements. We also ran a NMDS to visualize collective response of body shape divergence. ANOVAs revealed that 7 out of 13 morphometric measurements were significantly different among populations (P < 0.05) and NMDS resulted in three distinct non-overlapping clusters for three populations (R² = 0.989; Stress = 0.111). Collectively, our results suggest that both translocated fish populations rapidly diverged to produce a more robust body shape with a short and deep caudal peduncle.

DRAGONFLY PERCH SELECTION RELATED TO PERCH HEIGHT AND LOCATION. Jessica L. Beard & Deborah Waller, Dept. Biology, Old Dominion University, Norfolk, VA 23529. Male dragonflies patrol territories to secure food and mates and rest on perches. Communities of dragonflies at four different lakes were studied for perch selection based on height and distance from the shore during the summer of 2012. Perch distance from shore had no effect on dragonfly perch choice. Tall perches (90 cm) were chosen most frequently by Libellula incesta, Libellulla needhami, and Celithemis eponia. Short perches (30 cm) were chosen most frequently by Brachymesia gravida and Erythemis simplicicollis. Pachydiplex longipenis perched on both tall and short perches. Perch choice was not related to dragonfly size. P. longipenis, which was the most common dragonfly at all four lakes, won more interspecific contests on tall perches and lost more interspecific contests on short perches. Overall, the amount of time spent on perches was less than one minute on all perches for all species.

THE EFFECT OF GROOMING BY HUMANS ON SOCIAL AND SELF-GROOMING IN HORSES. E. Squires & D. Waller, Dept. of Biol., Old Dominion Univ., Norfolk, VA 23529. Grooming behaviors of primates have been extensively studied, but comparatively little research has been done on the grooming habits of other mammals. The domestic horse provides an interesting subject for studies on grooming behavior because of the role humans play in the grooming process. Domestic horses stabled by private owners generally have all of their grooming needs met by humans who brush and bathe them. In contrast, some domestic horses are free-ranging and have little to no physical contact with humans and take care of their own grooming needs. This study examined the grooming behaviors of domestic horses kept by private owners in a barn on the Eastern Shore of Virginia. These horses were observed in two rounds in the pasture when turned out with other horses; all grooming behaviors exhibited individually and in groups were recorded. In the first round, the horses were not groomed by a human for at least 48 hours prior to observation. In the second round, the horses were groomed within an hour prior to observation. Three sets of rounds, each with 22-32 horses, were conducted. Horses ungroomed by humans groomed themselves more frequently than horses recently groomed, but social grooming was not affected by human grooming. An enhanced understanding of equine grooming behavior will benefit animal behaviorists, private owners, and all other horse enthusiasts.

THE EFFECT OF ETHANOL ON BLOW FLY LARVAE AND PUPAE. Jessica Sterling & Deborah Waller, Dept. Biology, Old Dominion Univ., Norfolk, VA 23529. Insects are frequently used in forensic investigations to determine postmortem interval (PMI) and circumstances surrounding death. Entomotoxicology includes the investigation of the effects of drugs and toxins on arthropod development. Use of various drugs and toxins prior to death can affect maggot development rates on the corpse, resulting in inaccurate estimations of PMI. The objective of this study was to determine how ethanol affects larval and pupal stages of blow flics (Diptera: Calliphoridae). Cat food baits, which attract carrion feeding insects, were placed in wooded areas to collect blow fly eggs. No insects visited baits during the winter trials, but during the spring baits were colonized by carrion flies and also predators such as spiders. Blow fly larval developmental rates will be related to whether larvae were reared on control baits or baits with different concentrations of ethanol.

# Psychology

### Posters

FAMILY RESOURCES, ADHERENCE, AND GLYCEMIC CONTROL IN ADOLESCENTS WITH TYPE 1 DIABETES. Patrick J. Weaver<sup>1</sup>, Elizabeth M. Robinson<sup>1</sup>, Laura J. Caccavale<sup>1</sup>, Zachary Radcliff<sup>1</sup>, Rusan Chen<sup>2</sup>, Randi Streisand<sup>3,4</sup>. & Clarissa S. Holmes<sup>1</sup>, <sup>1</sup>Virginia Commonwealth University, <sup>2</sup>Georgetown University, <sup>3</sup>Children's National Medical Center, & <sup>4</sup>George Washington University. Objective: To examine the relations among family resources (family organization and marital status), adherence, and glycemic control in adolescents with type 1 diabetes. Methods: 257 parent-child dyads were recruited from two pediatric endocrinology clinics. Youth were 11 to 14 years of age (mean age = 12.8, SD = 1.2), primarily Caucasian (69.6%), 22.7% lived in single-parent households, and 77.5% in middle/upper-middle class homes (mean SES = 46.6, SD = 11.7). Results: Structural Equation Modeling revealed an indirect path among family resources, regimen adherence, and glycemic control (β=-0.21, p=.004). A direct relation between family resources and glycemic control was nonsignificant (β=-0.21, p=.071) and demonstrated full mediation of the effect of family resources on glycemic control through regimen adherence. Conclusions: Lower family resources are associated with poorer glycemic control through poorer adherence. Family organization is a modifiable component of family resources that could be the focus of interventions designed to enhance better adherence behaviors and glycemic control.

THE EFFECTS OF DOLL PLAY ON THE FEELINGS OF EFFICACY FOR FUTURE FAMILY AND OCCUPATIONAL ROLES. <u>E.T. Parrott</u>, K. M. Schroeder, & R. B. Dent, Department of Psychology, Washington and Lee University, Lexington, VA. 24450. The present study investigates how playing with dolls that have an amplified focus on a gendered body affect a child's gender typicality of play and a

child's feelings of efficacy for gendered skills and tasks. Children first acted out a story which they had been told by the researcher. They then had two free play sessions, one with the assigned doll and the other without. Lastly, participants answered questionnaires about their future occupation and family role. Though the current data was analyzed using hierarchical regression and no relationship was found between ratio scores of play types and future occupation or future family roles, data collection is still in progress. This study was funded in part by Washington and Lee University and the Virginia Academy of Science.

### Statistics

SYNCHRONY OF HETEROGENEOUS SPIKING NEURON MODELS. Cheng Ly, Department of Statistical Sciences and Operations Research, Virginia Commonwealth University, Richmond, Virginia 23298. Heterogeneity is a realistic physiological attribute neglected in many mean-field models in Neuroscience. To this end, we consider a coupled stochastic neural network model where each neuron has distinct or heterogeneous intrinsic properties. A proper description of this system is large dimensional and unwieldy, requiring reduced descriptions for tractability. We present some results of reduction methods to capture various statistical quantities of interest (i.e, level of synchrony).

STATISTICAL PATTERN RECOGNITION USING GAUSSIAN COPULA. <u>Sumen Sen</u> & Norou Diawara, Department of Mathematics and Statistics, Old Dominion University, Norfolk, Va 23529. Statistical pattern recognition is a field of study interested in classification accuracy and feature selection. Much significant research effort has been done because such problems have a vast area of applications in automatic character recognition, medical diagnostic. Classical discrimination theory assumes normality when calculating joint and conditional distribution and estimation of parameter models. However, such normality assumption is often questionable and quite restrictive. In some situations, the pattern vector is a mixture of discrete and continuous random variables. In this talk, we use copula densities to model class conditional distribution. These types of model structures are useful for a mixed pattern vector. We use simulation to compare the performance of the copula based classifier with classical normal distribution based model.

ESTIMATING SURVIVAL FUNCTIONS THROUGH MODEL COMBINING. Lihua Chen¹ & Panayotis Giannakouros², ¹Department of Mathematics and Statistics, James Madison University, Harrisonburg, VA 22801, ²Center for Computational Mathematics and Modeling, James Madison University, Harrisonburg, VA 22801. A model combining method is developed to estimate survival functions to account for model selection uncertainty. The weighting of models is based on the predictive performance of models. This weighting method has a connection with information theory which guarantees that up to an additive penalty term of order 1/n, the combined estimator performs as well as the best estimator in the model list. Empirical studies

demonstrate the advantage of this method. A theoretical risk bound on the combined estimator is also obtained.

EFFECTS OF ENVIRONMENTAL CHEMICAL MIXTURES ON RISK OF NON-HODGKIN LYMPHOMA. Jenna N. Czarnota, Chris Gennings, & David C. Wheeler, Department of Biostatistics, Virginia Commonwealth University, Richmond, VA 23298. Given that humans are exposed to a multitude of environmental chemicals simultaneously, it is of particular importance to examine the relationship between chemical mixtures and disease risk. Exposure profiles may change spatially, and thus it is also necessary to consider the impact of varying exposure patterns on the effect of a chemical mixture. A weighted quantile sum (WOS) approach was used in conjunction with non-linear logistic regression to model the association of a mixture of 27 correlated environmental chemicals measured in house dust and risk of non-Hodgkin lymphoma (NHL). The data were obtained from the National Cancer Institute Surveillance Epidemiology and End Results Program NHL case-control study. Analyses were performed overall (full data set) and locally (separately at each of 4 study sites), demonstrating differences in exposure, mixture effect, and relative importance of individual chemicals. Through simulation studies, the performance of WQS regression was examined in comparison to traditional shrinkage methods in terms of sensitivity and specificity in the selection of harmful chemicals.

ADJUSTING FOR COVARIATES USING PROPENSITY SCORE METHOD WHEN AUC IS USED AS MEASURE OF TREATMENT EFFECT. Hadiza Galadima & Donna McClish, Department of Biostatistics, Virginia Commonwealth University, Propensity score methods have been widely used in Richmond, VA 23298. epidemiologic research to reduce bias in cohort studies. For continuous outcomes, the mean difference between two risk groups is a well-known measure of group effect. Another effect measure for which there has been an increased interest in the literature is the probability that a randomly selected participant in the treatment group (X) has a better result than a randomly selected participant in the comparison group (Y), i.e. P(X>Y). This probability is equivalent to the area under the curve (AUC), a common measure used with receiver operating characteristic (ROC) curves to assess accuracy of medical tests. We use the method of stratification on the propensity score to estimate AUC while controlling for confounding. The adjusted AUC estimator is a weighted average of the stratum-specific AUCs. Furthermore, we provide confidence interval for the adjusted AUC. Finally, we compare the adjusted AUC with the well-known Mann-Whitney non-parametric statistic. We illustrate the methodology using a sample of adults with sickle cell disease (SCD), living in the Richmond and Tidewater areas of Virginia to estimate the effect of gender on frequency of pain due to SCD controlling for differences between groups.

PLANNING FATIGUE TESTS FOR POLYMER COMPOSITES. <u>Caleb King</u><sup>1</sup>, Yili Hong<sup>1</sup>, Stephanie P. DeHart<sup>2</sup>, & Patrick A. DeFeo<sup>2</sup>, <sup>1</sup>Department of Statistics, Virginia Polytechnic Institute and State University, Blacksburg VA 24061 &, <sup>2</sup>Applied Statistics Group, DuPont, Wilmington, DE 19803. In this paper, we present optimal designs for cyclic fatigue testing with the goal of minimizing the asymptotic variance of a lifetime percentile at a design stress level. The designs are based on a model adapted from the

fatigue literature that is derived from assumptions regarding damage accumulation in polymer composite materials. Specifically, this model is able to incorporate aspects of the testing procedure and is more suitable for modeling of cyclic fatigue in polymer composites than the model used in the current standards. We provide a comparison between our optimal designs and the traditional designs currently in use and propose a compromise design to combine the minimum variance with a suitable number of stress levels. The effects of the design and model parameters on the asymptotic variance are studied and suggestions for good designs are presented based on the results. A simulation study is used to compare the exact and asymptotic variances of the estimated lifetime percentile at the design stress level. Finally, we conclude with a summary of the results and provide some areas for future research.

MULTI-CHANGE POINTS DETECTION IN SINGLE INDEX MODEL. Hamdy F. F. Mahmoud', Inyoung Kim' & H. Kim<sup>2</sup>, 'Department of Statistics, Virginia. Polytechnic Institute and State University, Blacksburg VA 24061, <sup>2</sup>Department of Biostatistics and Epidemiology, School of Public Health, & Institute of Public Health and Environment, Seoul National University, Seoul, Republic of Korea. Environmental health studies are of great interest in human research to evaluate the relationship between mortality and temperature. It has been shown that there is a nonlinear relationship between these two variables with a fixed number of change points for temperature. Generalized linear models with log link (GLM) or generalized additive models (GAM) have been used to describe this nonlinear relationship. The currently available methods consist of two steps: they first estimate the models and then detect change points. However, the methods for simultaneously identifying the nonlinear relationship and detecting the number of change points are quite limited. To address these limitations, a unified approach is proposed for its ability to simultaneously estimate the nonlinear relationship and detect the change points. A single index change point model (SICM) is proposed as our unified approach. A permutation-based testing procedure is also provided to detect multiple change points. SICM is compared with GLM and GAM using simulation and a real application. Simulation results suggest that our approach performs better in terms of Type I error and power and outperforms in both model fitting and change points detection. The asymptotic properties of the permutation test are showed for SICM, suggesting that the number of change points is consistent. The advantages of our approach are demonstrated using the mortality data of Seoul, Korea from 2000 to 2007. Our approach detected two change points for temperature while other approaches found only one.

A TIME-VARYING PARAMETER STATE SPACE MODEL FOR THE NIGERIAN ECONOMY. Olushina Alawale Awe<sup>1</sup>, Ian Crandell<sup>2</sup>, & Scotland Leman<sup>2</sup>, <sup>1</sup> Department of Mathematics, Obafemi Awolowo University, Ife, Nigeria & <sup>2</sup> Department of Statistics, Virginia Polytechnic Institute & State University, Blacksburg, VA 24061. In recent years, state space models have gained tremendous popularity in statistics and econometrics especially in the modeling of time series data. In this paper, we present a time-varying parameter state space model for economic indicators using data from the Nigerian Economy. Economic indicators are mainly used for measuring economic trends. Policy makers in both advanced and developing nations make use of

economic indicators like GDP to predict the direction of aggregate economic activities. We apply the Kalman filter algorithm to obtain posterior inference on state space parameters specified from a Dynamic Linear Model (DLM), which implicitly describes the overall state of the economy. Upon estimating this new model using Nigerian economic data from 1960-2009, our model is able to detect outliers, structural breaks and historical trends in the time series considered. Our initial exploratory analysis indicates that traditional leading variables like money supply, exchange rate and capital expenditure are useful in forecasting the GDP, which is indicative of the long-run economic growth of Nigeria. Also, our method provides a full option that facilitates efficient and computationally less intensive posterior simulation through Markov chain Monte Carlo (MCMC) algorithms.

PENALIZED STEREOTYPE LOGIT MODEL FOR HIGH-DIMENSIONAL DATASETS. Qing Zhou & Kellic J. Archer, Department of Biostatistics, Virginia Commonwealth University, Richmond, VA 23298. Traditional methods for modeling ordinal data do not perform well in the presence of a high-dimensional covariate space, because traditional methods require that the number of samples is greater than the number of covariates and assumes covariate independence. The general monotone incremental forward stagewise method (GMIFS) was recently adapted to fit cumulative logit, adjacent category, and continuation ratio models, and were shown to be capable of deriving a parsimonious classifier (K Archer, J Hou). One limitation of these methods is that the proportional odds assumption may not hold for all genes in the dataset. Therefore in this paper, the GMIFS method was extended to the stereotype logit model to cope with situations when the proportional odds assumption does not hold. The method was applied to gene expression dataset to predict stage of disease.

LISA 2020: A SPARK FOR GLOBAL DEVELOPMENT. Emanuel Msemo1 & Eric Vance<sup>2</sup>, Department of Biometry and Mathematics, Sokoine University of Agriculture, Morogoro, Tanzania & <sup>2</sup>Department of Statistics, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061. For quality and significant results, researchers need to adopt methods of data collection and analysis that best suit their objectives. There is a need for researchers to collaborate with statisticians in the planning and analysis stages of research for better results. To increase the global impact of statistics, LISA (Virginia Tech's Laboratory for Interdisplinary Statistical Analysis) is partnering with universities and individuals around the world to implement its plan to create a network of 20 new statistical collaboration laboratories in developing countries by 2020. This plan is called "LISA 2020". It involves training statisticians from developing countries through a formal procedure. When the trained statisticians return to their home countries, they will establish a statistical collaboration laboratory and interact with other researchers to help solve real-world problems in statistical practice. In this talk, I will explain my involvement in LISA 2020 at Virginia Tech, and will touch upon the process of establishing a statistical laboratory at Sokoine University of Agriculture (SUA) in Tanzania. Through this laboratory, statisticians at SUA will collaborate with researchers and provide them with support and mentorship in statistical thinking, analysis and education so they will have the skills to turn their research into tangible results for Tanzanian farmers. Our belief is that well planned research accompanied by proper analysis of data is vital to improved agricultural productivity, which will impact the lives of Tanzanians and people throughout the world.

BIVARIATE DOUBLY-INFLATED POISSON AND RELATED REGRESSION. Pooga Sengupta & N. Rao Chaganty, Department of Mathematics and Statistics, Old Dominion University, Norfolk, VA 23529. Count data are common in observational scientific investigations and in many instances such as twin or crossover studies, the data consist of dependent bivariate counts. An appropriate model for such data is the bivariate Poisson distribution given in Kocherlakota and Kocherlakota (2001). However, in situations where inflated count of (0,0) occur, Lee, et al. (2009) proposed the zero-inflated bivariate Poisson distribution that accounts for the inflated count. In this research we introduced and studied a bivariate distribution that accounts for an inflated count of the (k, k) cell for some k>0, in addition to the inflated count for the (0,0) ccll. This bivariate doubly-inflated Poisson distribution (BDIP) is a parametric model determined by four parameters (p<sub>s</sub>). In this talk we will first discuss the distributional properties such as identifiability, moments and conditional distributions and stochastic representation of the BDIP model. Next we will discuss parameter estimation by the method of moments and maximum likelihood methods and a comparison of the methods via asymptotic relative efficiency calculations. If time permits we will also discuss the BDIP regression model that incorporates covariates into the BDIP model, and we will illustrate applicability of the BDIP regression model to analyze a subset of the Australian Health Survey data.

A ROBUST ESTIMATION OF SUFFICIENT DIMENSION REDUCTION. Qin Wang & Hossein M. Rekabdarkolaee, Department of Statistical Sciences and Operations Research, Virginia Commonwealth University, Richmond, VA 23298. Dimension reduction and variable sclection play important roles in high dimensional data analysis. MAVE (minimum average variance estimation) is an efficient approach proposed by Xia et al (2002) to estimate the regression mean space. However, it is not robust to outliers in the dependent variable because of the use of least-squares criterion. In this project, we propose a robust estimation based on local modal regression so that it is more applicable in practice. The efficacy of the new approach is illustrated through simulation studies and a real data analysis. Furthermore, we extend the new approach to select informative variables through shrinkage estimation.

INTRODUCING COVARIATES IN THE ATTRIBUTE-LEVEL BEST-WORST DISCRETE CHOICE MODELS. Amanda Working & Norou Diawara, Department of Mathematics and Statistics, Old Dominion University, Norfolk, VA 23529. Marketing research is interested in including covariates into the modeling of attribute-level best-worst discrete choice experiments. In the usual discrete choice experiments (DCE), the interest is to predict which product a consumer will choose. These types of experiments do not provide much information as to why the consumer chose the product. In an attribute-level best-worst DCE, a decision maker is provided profiles comprised of attribute-levels for each attribute describing a certain type of product and is asked to pick the pair of attribute levels that contains the best and worst. The usual models for attribute-level best-worst discrete choice experiments depend only on the attributes and attribute-levels. In the model we present, we still model the choice of

best-worst attribute-level pair, but we include covariates that are class depending. The class allocation is fitted under a mixed logit model. Such heterogeneous classification generalizes the choice impact of consumers. An example is developed and given as illustration

LISA 2020: CREATING A NETWORK OF STATISTICAL COLLABORATION LABORATORIES. Eric Vance, Department of Statistics, Virginia Polytechnic Institute and State University, Blacksburg VA 24061. To increase the global impact of statistics as a discipline useful for helping researchers answer research questions, and sponsored by a Google Research Award, LISA-The Laboratory for Interdisciplinary Statistical Analysis at Virginia Tech—is partnering with universities and individuals around the world to create a network of 20 new statistical collaboration laboratories in developing countries by 2020, LISA and its partners will educate and train statisticians from developing countries to communicate and collaborate with nonstatisticians and then support these statisticians to create statistical collaboration laboratories in their home countries to help researchers, government officials, local industries, and NGOs apply statistical thinking and data science to make better decisions through data. At LISA and elsewhere, we will unlock the collaborative potential of technically sound statisticians who will in turn unlock the research potential of their collaborators and teach other statisticians to do likewise. These local research collaborations, now with the power of statistical thinking and data science open to them, will be key to improving human welfare worldwide. This talk will focus on the steps of the LISA 2020 plan, including how LISA trains statisticians to become interdisciplinary collaborators, how statistical collaboration laboratories create knowledge, and how we are building a mentoring network to assist statisticians in developing countries to enable and accelerate research by making statistics more practical in solving real world problems.

# Structural Biology, Biochemistry, and Biophysics

EVOLUTION OF THE SYNUCLEIN PROTEINS: ORPHANS OR SUPERFAMILY MEMBERS. Lesley Greene, Agatha Munyanyi, John Bedford, & Zeinab Haratipour, Department of Chemistry and Biochemistry, Old Dominion University, Norfolk, VA 23529. The synucleins are composed of three related proteins:  $\alpha$ ,  $\beta$  and  $\gamma$ . The functions and structures of these proteins in the brain remain unresolved. Computational approaches such as bioinformatics can be highly informative and advance theories about the structure and function of proteins long before lengthy experimental work is completed. Computational research can also inform and direct experimental directions. In line with this perspective we conducted bioinformatics and modeling research to facilitate understanding the structure and function of the synucleins. Using PSI-BLAST searches we identified four proteins which contain large regions of significant sequence identity and similarity with the synucleins. These proteins are an endoglucanse enzyme from Acetorbacter pomorum, a CRE-DUR-1 protein from a nematode, a cytochrome c from a spiral bacterium and a putative protein from the Tasmanian Devil, Three-dimensional molecular modeling indicates that these proteins share a common helix-turn-helix structure with the membrane-bound form of the α-synuclein monomeric NMR structure. This data and their evolutionary status will be presented. We thank Old Dominion University for funding to LG and from the American Association of University Women to AG for this work.

NEW FUNCTIONS OF FLAVIN DEPENDENT ENZYMES: THE MECHANISM OF 2-HALOACRYLATE HYDRATASE. Yumin Dai & Pablo Sobrado, Department of Biochemistry, Virgina Polytechnic Institute & State University, Blacksburg, VA, 24061. The flavin-dependent 2-halocrylate hydratase (2-HAH) catalyzed conversion of 2-chlorocrylate, a major component in manufacturing of acrylic polymers, to a nontoxic pyruvate through a double bond hydration and subsequent chloride elimination. The enzyme from Pseudomonas sp. YL has been cloned, recombinantly expressed and purified from E. coli. The enzyme, which is monomer in solution, contains FAD and is active only in the reduced state, although the reaction is redox neutral. Enzyme activity was recorded after incubation of the enzyme with 2 mM NADH under light for 10 min ( $k_{cat} = 1.35 \pm 0.02 \text{ s}^{-1}$ ;  $K_M = 2.01 \pm 0.19$ ). Interestingly, reaction with NADH is slow in the absence of light. When the enzyme is exposed to light, the reduction occurs ~ 30 fold faster, indicating the light activation is unique to 2-HAH. Mechanism of 2-HAH was studied, and the results indicated FAD radicals, both neutral and anionic semiquinones were involved during the catalysis. This work was supported by NIH grant R01 GM094469.

STRUCTURAL BASIS OF PHOSPHOINOSITIDE (PIP) RECOGNITION BY THE TIRAP PIP-BINDING MOTIF. Xiaolin Zhao, Shuyan Xiao, & Daniel G. S. Capelluto, Department of Biological Sciences, Virginia Tech., Blacksburg VA 24061, TIRAP is an adaptor molecule required for both plasma membrane and endosome TLR signaling in response to viral infection. TIRAP contains a TIR domain, which is responsible for association with another adaptor protein, MyD88; and a N-terminal PIP binding domain (PBD) that allows TIRAP's promiscuous binding to various lipid targets, such as phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>) at the plasma membrane and phosphatidylinositol 3-phosphate (PI<sub>3</sub>P) on the endosomes, resulting in functional formation of myddosome in both locations. A minimum region (residues 15-35) in PBD is sufficient to associate to plasma membrane. We show that this region, we called the PIP-binding motif (PBM) adopts a helical conformation in the presence of artificial membrane, such as dodccylphosphocholine micelles, and more importantly, monodispersed PIP, and PI,P can induce helical structure of TIRAP PBM, but neither monodispersed phosphatidylinositol nor inositol trisphosphate (head group of PIP, IP,) induce TIRAP PBM's helical structure. Resonance assignments of TIRAP PBM NMR spectra reveal that conserved basic residues Lys16, Lys31, and Lys32 play important roles in association with PIPs at either plasma membrane or endosome. Mutagenesis studies identified key-PIP, interacting residues of TIRAP PBM. We are in the process to precisely identify the PIPs binding site in TIRAP PBM and to obtain the solution structure of it in the free and PIP,-bound states to understand the progressive mechanism and regulation of membrane association of the protein.

STRUCTURAL STUDIES OF A DUAL-DOMAIN SYSTEM FROM THE GIANT MUSCLE PROTEIN OBSCURIN (Ig58/50). N. T. Wright, L. C. Meyer, M. C. Oehler & T. A. Caldwell, Dept. of Chemistry and Biochemistry, James Madison University.

Obscurin is a giant protein (800-900 kDa at its largest) that serves to link the contractile apparatus to the surrounding membrane structure in muscle cells. This protein is highly modular in nature, and mostly consists of tandem Ig domains. Each of these domains has a unique sequence, and each domain that has been extensively studied has unique binding partners. Two of these domains- Ig58and Ig59- are both required to bind to the giant muscle protein titin at ZIg9 and ZIg10. An R->Q mutation in the obscurin Ig58 domain is associated with cardiomyopathy, presumably through disruption of obscurin/titin binding. Here, we present the high resolution structures of Ig58 and Ig59. Both domains fold in a typical Ig fold, but Ig58 has an extensively charged surface. NMR analysis shows that no structural deformations occur with the R4344Q mutation, however MD simulations indicate that the surface electrostatic interactions change significantly, SAXS data and NMR data suggest that Ig58 and Ig59 do not extensively interact in solution. These findings suggest a molecular mechanism of how obscurin interacts with its targets.

OLIGOMERIC STATES OF AN ADENOVIRUS RECEPTOR, CD46, DIFFER DUE TO ALTERNATIVE SPLICING. Emily L. Romanoff & Eugene Y. Wu, Dept. of Biology, 28 Westhampton Way, University of Richmond, Virginia 23173. CD46 is an integral membrane protein expressed on the surfaces of many human cells. CD46 is alternatively spliced into BC (~60-65 kDa) and C (50-55 kDa) isoforms; the BC isoform contains an extra serine-threonine-proline rich (STP) domain that is highly Olinked glycosylated. Adenovirus type 37 (Ad37), a species D adenovirus, can use the C isoforms of CD46 as a receptor to cause ocular and genital tract infections, but Ad37 does not bind A549 lung epithelial cells efficiently. Based on existing structural data. we hypothesized that the extra B STP domain of CD46 could prevent oligomerization at the cell surface. To explore whether adenovirus tropism is linked to CD46 isoform expression, we treated HeLa cervical carcinoma cells, which are known to express both BC and C isoforms, with a chemical crosslinker that does not cross the cell membrane. Western blotting of the cell lysate showed no substantial change in the migration of the BC isoforms of CD46 after crosslinking, but the C isoforms exhibited a large change in molecular weight to > 250 kDa, Western blotting of crosslinked A549 cells, which only express the 60-65 kDa isoforms, also showed no substantial change in molecular weight of CD46. These results indicate that alternative splicing of CD46 produces two different extracellular domains that can be differentiated by their oligomeric states. The absence of CD46 C isoforms on lung epithelial cells correlates with low binding and infection by Ad37, which may preferentially bind oligomeric CD46. These results may have implications for the tropisms of many human pathogens that use CD46 as a receptor.

IONOTROPIC GLUTAMATE RECEPTOR DYNAMICS. <u>D. Holley</u><sup>1</sup>, S. Holmes<sup>1</sup>, A. Hahn<sup>2</sup>, & C. A. Parish<sup>1</sup>, <sup>1</sup>Department of Chemistry, University of Richmond, Richmond VA 23173 & <sup>2</sup>Harvard School of Dental Medicine, Harvard University, Boston, MA 02115. Ionotropic glutamate receptors (iGluRs) are tetrameric, ligand-gated ion channels that initiate post-synaptic, fast, excitatory neurotransmission in the mammalian central nervous system. The gating mechanism involves a conformational change that arranges the transmembrane region into a pore, allowing ions to move across the neuronal membrane. Glutamate, which is released as a neurotransmitter by

pre-synaptic neurons, induces this conformational change by binding the iGluR ligandbinding domain (LBD), forming bonds that stabilize the clamshell-like structure of the LBD into a sustained, occluded conformation. The ligand-induced, sustained closure of the LBD may instigate the pore-forming conformational change in the transmembrane domain. Understanding LBD structural dynamics is a necessary step in deconstructing the mechanism that leads to glutamate binding. Here, we use 400 and 600 ns all-atom molecular dynamics (MD) simulations to measure the stability and inherent motions of the isolated, solvated, apo iGluR ligand binding domains for three iGluR subfamily-specific subunits; GluR2, NR2A, and NR2B. As no crystal structures are available for NR2B, we constructed a homology model based on NR2A sequence similarity (PDB code 2A5S). We show that a majority of the large-scale LBD dynamics can be described by three different eigenvectors, and that the first eigenvector correlates to the opening and closing of the LBD, which we estimate occurs every 60-150ns. Our results also indicate that LBD hinged movement may be constrained by LBD dimer interactions, suggesting that the flexibility of the isolated LBD monomer reported in several recent MD studies may in part represent an artifact of monomeric systems.

GLUTAMATE INDUCED CHANGES IN CONFROMATIONAL FLEXIBILITY IN THE LIGAN BINDING DOMAIN IONOTROPIC GLUTAMATE TRVR[YPTD. Philip Varnes, Forest Barkdoll-Weil, Carlos Metz, & Ellis Bell, Chemistry Dept., University of Richmond, Richmond, VA. Ionotropic Glutamate receptors (iGluRs) are tetrameric proteins with 4 domains, an amino terminal domain, the ligand binding domain, the transmembrane ion channel and a largely disordered intracellular C terminal region. The ligand binding domain binds glutamate, a major fast excitatory neurotransmitter, which activates the receptor channels. There are three main families of iGluRs, AMPA, NMDA, and kainite which among other things differ in their responses to two endogenous neurosteroids, pregnenalone sulfate (PS) and pregnanalone sulfate (PREGAS). To investigate the role that conformational flexibility of the ligand binding domain plays in response to either glutamate or the neurosteroids (in the presence or absence of glutamate) we have used Multiwavelength Collisional Quenching (MWCQ), a novel approach to collisional quenching using multiwavelength analysis of spectral data collected at two different excitation wavelengths which reports on the exposure and charge environment of tyrosines and tryptophans in the protein. While Glutamate has different effects on flexibility depending upon the family of receptor, the use of differently charged quenching molecules shows glutamate induced changes in the charge environment that can be correlated with steroid binding ability. This work was supported by NSF Grants MCB-104995 and MCB 0448905 to EB.

A COMPUTATIONAL MODEL FOR THE IRON HOMEOSTATIS IN E. Coli. Ovidiu Lipan, Dept. of Physics, Univ. of Richmond, Richmond VA, 23173. In order to establish an infection in a human organism, bacteria must grow and multiply effectively. Bacteria need iron for growth and for that reason a homeostatic system evolved to control iron acquisition and the intracellular iron levels. Iron is one of the important factors intimately connected to the synthesis of some of the virulence determinants. E. Coli bacteria are highly used as an experimental model for understanding the adaptation of bacteria to different iron environments. A couple of deterministic models for the iron homeostatic system appeared in the literature.

However they do not incorporate the abrupt probabilistic discontinuities that appear at the end of the bacteria's cell cycle. At division, the number of molecules is split between the mother and the daughter cell. Also, the moment in time for division is not strictly periodic and depends on the maturity of the bacteria. Following an idea of von Foerster and Rubinow we present a computational model for the Iron homeostatic system in E. Coli that combines a deterministic approach for the regulatory system with a probabilistic model for the bacteria's growth and division processes. We present the method to obtain the partial differential equation and a numerical approach to solve for the time evolution.

EXPLORING THE STEROID BINDING SITE OF IGLURS BY LIMITED PROTEOLYSIS AND HYDROGEN DEUTERIUM EXCHANGE. Emily Bartle & Ellis Bell, Dept. of Biochemistry, University of Richmond, Richmond VA 23060. AMPA, NMDA, and kainate receptors belong to the ionotropic glutamate (iGluR) family of receptors located in the post-synaptic neural membrane. These receptors bind glutamate, a major fast excitatory neurotransmitter, which activates the receptor channels. Consequently, the receptor's proper function plays an important role in synaptic plasticity, memory and learning. Mis-regulation of these receptors has been linked to many neurodegenerative diseases, such as Alzheimer's and Parkinson's disease. Thus, our research focuses on understanding the receptors' activity and regulation to ultimately develop therapeutic applications. Additionally, two endogenous neurosteroids, pregnenalone sulfate (PS) and pregnanalone sulfate (PREGAS), are known to contribute to regulating the potentiating and inhibiting effects of iGluRs on neuronal activity. Therefore, the primary aim of this branch of the project is to determine the exact locations for the binding sites of these two neurosteroids to the receptors. Using molecular docking studies and other computational methods, several potentially significant residues have been suggested by our collaborator and subsequently tested via sited directed mutagenesis. Because the site directed mutagenesis has yet to produce significant changes in binding affinity, we are attempting to answer the same question through alternative methods, such as limited trypsin proteolysis and hydrogen/deuterium exchange protection assays.

INVESTIGATION INTO THE OPENING MECHANISM FOR DNA POLYMERASE USING MOLECULAR DYNAMICS SIMULATIONS. Bill R. Miller III<sup>1,2</sup>, Carol A. Parish<sup>2</sup> & Eugene Y. Wu<sup>1</sup>, <sup>1</sup>Dept. of Biol., Univ. of Richmond & <sup>2</sup>Dept. of Chem., Univ. of Richmond, Richmond VA 23173. Crystal structures of DNA polymerase, a key enzyme for DNA replication in organisms, have shown an important α-helix on the enzyme, called the O-helix, plays a crucial role in binding the incoming dNTP. The O-helix can form an "open", "ajar", and "closed" conformation in the presence or absence of certain substrates in the active site. Although crystal structures have given insight into these conformations, traditional biochemistry experimental techniques are unable to capture the details of the transition between these states and how the polymerase traverses the potential energy surface from open to closed and back again. In this study, we have utilized recent computational advances to simulate the opening of the fingers domain starting from the closed and ajar conformations of Bacillus stearothermophilus DNA polymerase Klenow fragment using dynamics on the microsecond time scale, and also simulated the open state for comparison. We have fully characterized the opening

process that occurs after catalysis, and determined the key events and movements that are critical to O-helix opening. The transition from ajar to open is quick (<20 ns), while the full transition from closed to open was observed taking nearly 300 ns. We observed a key intermediate step in the pathway from closed to open involving a salt bridge between an arginine side chain on the O-helix and an aspartate in the thumb domain. These simulations aid in the elucidation of the O-helix opening mechanism for DNA polymerase on an atomistic level not currently available with experimental measures.

EXAMINING THE IMPACT OF MUTATIONS IN THE X-LINKED MENTAL RETARDATION PROTEIN CASK. Leslie E. W. LaConte, Vrushali Chavan, Jeffrey Willis, & Konark Mukherjee, Virginia Tech Carilion Research Institute, Roanoke VA 24016. Mutations in the protein CASK (calcium/calmodulin-activated Scr-Thr kinase) are associated with a range of severe phenotypes, including pontoccrebellar hypoplasia, microcephaly, optic nerve hypoplasia, intellectual disabilities, and autistic chracteristics. CASK is widely expressed, but is most highly expressed in the central nervous system. Although a number of roles have been proposed (scaffolding, trafficking, transcription factor, kinase), no single function has been definitely identified as responsible for the severe phenotypes, although a CASK hypomorph mouse phenocopies the human condition quite well. A careful phylogenetic, structural, computational, and in vitro analysis of five known pathogenic mutations of CASK reveals that in high conserved regions, mutations are structural, whereas in less conserved regions, mutations are functional (less likely to lead to protein unfolding). Predicting the pathogenic nature of a sequence variant remains challenging to do computationally, but a simple cell-based assay helped quickly identify CASK mutations that led to protein misfolding and aggregation. This aggregation was reversible with the addition of glycerol to cell culture. More detailed future studies of CASK mutations will offer further insight into the function of CASK.

INTRINSIC DISORDER IN ADAPTOR PROTEINS INCREASES COMMITMENT TO ENDOSOMAL PROTEIN TRAFFICKING. Daniel G. S. Capelluto, Dept. Biol. Sci., Virginia Tech, Blacksburg VA 24061. Adaptor proteins are often committed to cellular processes that involve cargo internalization from the plasma membrane. Ubiquitinated cargo is internalized by endocytosis and delivered to early endosomes via intracellular vesicles. Adaptor proteins, such as Tollip and Tom1, facilitate cargo sorting through their ubiquitin-binding domains. Tollip is localized to early endosomes, through binding to phosphatidylinositol 3-phosphate (PtdIns(3)P). Tom1 can also bind ubiquitin-conjugated cargo and is recruited to the endosomal membranes through its association with Tollip. In this work, we demonstrate that binding of Tollip to PtdIns(3)P is negatively modulated by interaction with Tom1. Structural studies determine that the Toml-binding domain (TBD) of Tollip is intrinsically disordered and folds upon binding to the Toml GAT domain, which also undergoes a conformational change upon binding. Intermolecular NOEs of the Tollip TBD-Tom1 GAT complex indicate that association is mainly driven by hydrophobic contacts with high affinity. Ubiquitin binds to the Toml GAT domain at a site that does not overlap with that for the Tollip TBD, allowing their simultaneous binding. Using fluorescence microscopy, we show that mutations in the binding interphase of the Tom1 GAT and Tollip TBD complex leads to a dissociation of the proteins and triggers cytosolic

localization of Tom1. Accordingly, we propose that association of Tom1 to Tollip helps to release Tollip from endosomal membranes, allowing Tollip to commit to endosomal ubiquitinated cargo trafficking.

### Posters

DISABLED-2 MODULATES ERYTHROCYTIC INTERACTIONS THROUGH ITS SULFATIDE BINDING DOMAIN. Kaitlyn J. Andreano<sup>1</sup>, Mi Song Kim<sup>1</sup>, Daniel G. S. Capelluto<sup>2</sup>, & Carla V. Finkielstein<sup>1</sup>, <sup>1</sup>Integrated Cellular Responses Laboratory, <sup>2</sup>Protein Signaling Domains Laboratory, Department of Biological Sciences, Virginia Polytechnic Institute and State University, Platelets' role in cancer progression and metastasis has largely been attributed to platelet-mediated enhancement of tumor cell survival, extravasation, and angiogenesis. Correlations exist between the tumor cells' ability to aggregate platelets in vitro and their metastatic potential in vivo which is manifested as a hypercoagulable state found in most cancer patients. We have identified a tumor suppressor molecule (Dab-2) that is released upon platelet activation and that modulates the extent of blood clotting. Our results show Dab-2 is released from alpha-granules in the platelet to the membrane surface where it binds to the integrin receptor, thus inhibiting platelet aggregation. Binding of Dab-2 to integrins is modulated by sulfatides, a glycosphingolipid that accumulates in platelet membrane and effectively competes for Dab-2 levels. We found that Dab-2's N-terminal region binds to sulfatides through two conserved sulfatide-binding sites. Upon activation, sulfatides protect Dab-2 from thrombin cleavage, facilitate its internalization and modulate the surface expression of P-selectin, a coagulation protein needed for stabilization of platelet aggregates. P-selectin also mediates tumor cell adhesion to vascular endothelial cells and the interaction between activated platelets and cancer cells (cmboli) during metastasis. Our data show that Dab-2/sulfatide recognition influences the stability of platelet aggregates heterotypically with cancer cells through sulfatide binding and, indirectly, by controlling P-selectin levels.

RECOGNITION MAPPING OF BIMOLECULES ON SURFACES USING ATOMIC FORCE MICROSCOPY. Congzhou Wang & Vamsi K. Yadavalli, Department of Chemical and Life Science Engineering, Virginia Commonwealth University. Atomic force microscopy based adhesion force measurements have emerged as a powerful tool in the biophysical analysis of biological systems. Such measurements can now be extended to mapping biomolecules on biosurfaces via integrated imaging and force spectroscopy techniques. Critical to these experiments is the choice of the biomolecular recognition probe. We demonstrate how oligonucleotide aptamers can be used as versatile probes to image and spatially locate specific targets on surfaces. The unique advantage of this technique is its capability of localizing specific biomolecules via simultaneous topographic and force information in a short time. We focus on two structurally distinct proteins - human alpha thrombin and vascular endothelial growth factor (VEGF165) relevant to the clotting cascade. Via AFM-recognition mapping using their specific DNA aptamers, a clear consistency between height and force map obtained simultaneously on same areas is shown. The aptamer specificity and ability to distinguish their targets is shown through positive and negative controls. In addition, changes in binding due to blocking by free heparin shows the ability to study

fluctuating biological systems in real time. These two channels may be overlaid to form high resolution maps to identify proteins at the single molecule level. These results show that this approach has the capability to detect the presence of specific biomolecules which can increase our understanding of the fundamental organization, mechanics, interactions and processes on complex surfaces such as cells.

ASSESSING STUDENT UNDERSTANDING OF FOUNDATIONAL CONCEPTS OF PROTEIN STRUCTURE AND FUNCTION. Ellis Bell, Department of Chemistry, University of Richmond, Richmond, VA. A number of reports emphasize the importance of student understanding of foundational concepts and core competencies in the context of both disciplinary and interdisciplinary content and skills. For biochemistry and molecular biology, the content knowledge, skills and allied fields necessary for the discipline have been discussed in recent papers. Protein Structure and Function was identified as one of the five essential content areas. Principles of scientific teaching and backward design that have been used to align six critical components of understanding protein structure function relationships with focused assessment of student understanding and potential teaching strategies are illustrated. Of the six, two align with key features of understanding structure (Bonding & Dynamics), two with understanding biological function (Catalysis & Regulation), while two (Interactions & Evolution) clearly bridge the other concepts. Integrated with these core concepts of protein structure and function are the essential interdisciplinary concepts of Modularity, Energy, Change over time, Stochasticity and the Use of Mathematical models. Using a modified Bloom's taxonomy potential, assessments aligned to each concept of protein structure and function are discussed. In addition to assessing student understanding, the goal of such assessments is to incorporate key aspects of both the allied fields and the skills necessary for student success in biochemistry and molecular biology. Finally, potential student centered teaching strategies for introductory, gateway or capstone courses will be presented. This work was funded by NSF RCN-UBE Grant 0957205 to EB,

INVESTIGATION OF ENZYME KINETICS OF N-TERMINAL RCC1 PROTEIN METHYLTRANSFERASE REVEALS A DISTRIBUTIVE RANDOM ORDERED BI-BI MECHANISM. Stacie L. Richardson<sup>1</sup>, Gang Zhang<sup>1</sup>, Pahul Hanjra<sup>1</sup>, Yunfei Mao<sup>1</sup>, Darrell L. Peterson<sup>2</sup>, & Rong Huang<sup>1</sup>, <sup>1</sup>Department of Medicinal Chemistry & <sup>2</sup>Department of Biochemistry and Molecular Biology, Virginia Commonwealth University, Richmond VA 23219. Human protein N-terminal RCC1 methyltransferase 1 (NRMT1) methylates the N-terminal alpha-amino group of its protein substrates. This is the first example of a mammalian methyltransferase responsible for protein Nterminal methylation. NRMT1 is overexpressed in several types of cancer, including gastric, breast, head, and neck cancers, making it a potential therapeutic target. To understand its kinetic mechanism, we developed an assay for direct ratiometric quantitation by MALDI mass spectrometry. We have characterized the kinetic mechanism of recombinant NRMT1 using a MALDI-MS method for monitoring the various levels of methylation of its substrate peptides. We found that NRMT1 proceeds via a random ordered bi bi mechanism through Lineweaver-Burk analysis of the inhibitory patterns of the natural and dead-end products. Results of the processivity studies indicate that NRMT1 proceeds via a distributive mechanism for multiple

methylations. Based on our results, a bisubstrate inhibitor that mimics the structure of the ternary complex could be an effective selective inhibitor of NRMT1. In our ongoing studies we will investigate the inhibitory activity and mechanism of inhibitors designed in our lab against NRMT1 and its substrates.

INVESTIGATION OF SUPRESSOR OF IKK-EPSILON FUNCTION THROUGH STRUCTURAL ANALYSIS. Sean W. McKinley & Jessica K. Bell<sup>2</sup>, Department of Microbiology & Immunology, <sup>2</sup>Department of Biochemistry & Molecular Biology, Virginia Commonwealth University, Richmond, VA 23298. During viral infections, Toll-like receptor 3 (TLR3) stimulation initiates signaling to activate transcription of pro-inflammatory cytokines and type-1 Interferons, Suppressor of IKKE (SIKE) interacts with two kinases in the signaling pathway, IKKE and TANK binding kinase 1 (TBK1), inhibiting the transcription of type I interferons. Recently, we discovered that SIKE blocks TBK1-mediated activation of type I interferons by acting as a high affinity, alternative substrate of TBK1. To further characterize SIKE's function within the antiviral response, this study focused on defining the overall SIKE structure through X-ray crystallography. Using recombinant protein expressed from E. coli and purified via immobilized metal affinity chromatography, SIKE crystals were obtained from a sample concentrated to 15 mg/ml under several crystallization conditions. Yet, reproducing these results has been difficult. In this study, we have modified the purification scheme to remove an E. coli contaminant, SlyD. Purification under denaturing conditions, native conditions, incorporation of ion exchange and different IMAC resins has been tested. For each scheme, size exclusion chromatography and SDS-PAGE/Coomassie/silver stain were used to assess purity. Crystallization trials for samples from each purification scheme were completed. Supported by CCTR Endowment Fund.

USING COLOCALIZATION ASSAYS TO DEFINE SUPRESSOR OF IKK-EPSILON FUNCTION. Kenneth F. Lawrence<sup>1</sup> & Jessica K. Bell<sup>2</sup>, <sup>1</sup>Department of Microbiology & Immunology, <sup>2</sup>Department of Biochemistry & Molecular Biology, Virginia Commonwealth University, Richmond, VA 23298. The innate immune system is the body's first line of defense against pathogens. Signaling pathways initiated by pattern recognition receptors, such as Toll-like receptor 3 (TLR3), communicate the danger signal recognized by the receptor into the cell to mediate a rapid host defense. Suppressor of IKK-epsilon (SIKE) functions downstream of TLR3. Initially identified as an inhibitor of the TANK binding kinase 1 (TBK1)-mediated interferon response, we discovered that SIKE is a substrate of TANK-binding kinase 1 (TBK1). Co-immunoprecipitation assays of SIKE from epithclial and myeloid cells lines were analyzed by tandem mass spectrometry to identify interaction partners. These studies suggested that the SIKE interaction network impinged upon the cytoskeleton and RNA transport. We hypothesized that SIKE links TLR3 signaling to formation/resolution of RNA granules. To further delineate SIKE's role in RNA transport and cytoskeletal rearrangements, we used immunofluorescence assays to identify colocalization between SIKE and cellular markers in epithelial and myeloid cell types. Colocalization was quantified either at the level of whole cell or region of interest. SIKE was found to colocalize with cytoskeleton components (actin and tubulin), endosomal and plasma membrane markers (Rab11, LAMP-1, and LC3), and

ribosomes (S6). These results are consistent with our hypothesis that SIKE plays a role in trafficking related to the anti-viral innate immune response. Supported by CCTR Endowment Fund and NIH R21AI107447.

DESIGN, PREPARATION, AND VALIDATION OF AN ACTIVITY-BASED PROBE OF PROTEIN KINASE A. Robert A. Coover & Keith C. Ellis, Dept. Med. Chem., Va. Commonwealth Univ., Richmond VA 23298. Activity-based protein profiling is an important technique for analyzing the activity of proteins in disease states and validating their pharmacological modulation. The cAMP-dependent protein kinase (PKA) is the prototype kinase and its activity is regulated by multiple endogenous mechanisms, which include phosphorylation, binding to regulatory domains, localization by anchoring proteins, and redox regulation by glutathiolation. These mechanisms directly compete with or reduce substrate binding and/or modulate the conformation of the activation loop, a key motif in PKA activity. Using the crystal structure of PKA, we have developed a selective peptidomimetic covalent probe of PKA that utilizes high affinity portions of the PKI pseudosubstrate inhibitor. PKI is a pseudosubstrate inhibitor that preferentially binds to PKA when the activation loop is ordered and the enzyme is not bound to the regulatory subunit. We have prepared fluorophore-labeled PKI analogues that contain a halomethyketone at the C-terminus (P+2 position). We have evaluated our probe for specificity of labeling (confirmed by gel and MS), selectivity (lysates), and preliminary activity-based experiments and will present these data accordingly. This study was funded in part by the American Cancer Society Institutional Research Grant IRG-73-001-37 and the VCU Massey Cancer Center.

MOLECULAR DYNAMICS STUDIES OF IONOTROPIC **GLUTAMATE** RECEPTORS. Stephen Holmes, Dave Holley, Alex Hahn, Lisa Gentile, & Carol Parish, Department of Chemistry, Gottwald Center for the Sciences, University of Richmond, Richmond, VA 23173, Ionotropic glutamate receptors, iGluRs, are a family of ligand gated channels located in the post-synaptic neural membrane. These receptors serve a major role in the excitation of the neural cells, which is important for all neurotransmissions in the central nervous system. There are three subfamilies of iGluRs: NMDA, AMPA, and kainite receptors, NMDA receptors mediate the fast component of the excitatory post synaptic potential, AMPA receptors mediate fast synaptic transmission in the central nervous system. Kainate receptors have a major role in excitatory neurotransmission. A conformational change that opens a membrane pore is caused by the binding of the neurotransmitter glutamate to the extracellular ligand binding domain on these receptors, allowing cations to flow into the postsynaptic neural cell. Because glutamate functions as a major excitatory neurotransmitter in the central nervous system, the level of activity of iGluRs is strictly controlled. Neurodegenerative diseases such as schizophrenia and Alzheimer's, Parkinson's, and Huntington's diseases have been linked to mis-regulation of glutamate. Long time scale molecular dynamics studies using molecular dynamics were performed on a homology model for the S1S2 domain of the NMDA iGluR receptor. The resulting stable trajectories were subjected to exhaustive analysis. Dynamical results will be presented that shed light on the structure and function of these glutamate receptors.

X-RAY CRYSTALLOGRAPHY & SMALL ANGLE X-RAY SCATTERING STUDIES OF INTERFERON REGULATORY FACTOR 4. Soumya Govinda Remesh & Carlos R. Escalante, Department of Physiology & Biophysics, Virginia Commonwealth University, VA 23298-0613 USA. Interferon (IFN) regulatory factor family member (IRF4) is a transcription factor that serves specific roles in transcriptional regulation of IFN responsive genes is limited to the immune system. Generally, members of IRF family, like IRF3 and IRF5, have carboxy terminal autoinhibitory regions that are phosphorylated to generate a transcriptionally active dimer. IRF4 also has a carboxy terminal auto-inhibitory region but it gets activated by binding to multiple different partners. We have determined the crystal structure of the Cterminal activation domain of IRF4 and carried out small angle X-ray scattering (SAXS) studies to generate ab initio models for the full-length protein to obtain insights into the autoinhibitory mechanism. The data suggests that the putative linker of IRF4 connecting the N- and C-terminus is most likely a folded well-structured domain that interacts with the auto-inhibitory carboxy tail indicative of a different mechanism of activation for IRF4 compared to the other members of the family. We are currently trying to further our understanding of the activation process for IRF4 that will aid in highlighting its uniqueness within the IRF family. Information obtained by the current study will help in development of novel therapeutics in disease states that are mediated by IRF4 like multiple myeloma, cardiac hypertrophy and certain autoimmune diseases.

ASSEMBLY OF AAV2 REP68 ON AAVS1 DNA REQUIRES COOPERATIVITY OF INDIVIDUAL DOMAINS AND OLIGOMERIZATION. Clayton M. Bishop, Francisco Zarate-Perez, Faik N. Musayev, & Carlos R. Escalante, Department of Physiology and Biophysics, Virginia Commonwealth University, 1101 East Marshall Street, Richmond, VA 23298-0551 USA. Multiple DNA transactions are at the center of almost all processes regulating AAV life cycle. A common feature shared by all transactions is the binding of the large AAV Rep proteins Rep78/Rep68 onto DNA sites harboring multiple GCTC repeats. AAV mediated site-specific integration is contingent upon the formation of a productive complex between Rep78/Rep68 and the AAVS1 site located at chromosome 19. In order to understand the mechanistic details of the initial assembly process we carried out equilibrium binding experiments of Rep68 and its individual domains with a 42-mer AAVS1 site. Results show that although Rep68 binds AAVS1 with high affinity (69 nM), both the OBD and helicase individual domains bind DNA weakly with affinities of >>60μM and 22μM respectively under our experimental conditions. Mutant Rep68 proteins that have a defective oligomerization interface bind DNA poorly suggesting that productive binding requires both the concerted interaction of the individual domains with DNA and oligomerization. Moreover, we show that a minimal number of two repeats is required to form a stable complex.

STRUCTURAL AND FUNCTIONAL BASIS OF TOLLIP ASSOCIATION TO THE ENDOSOMAL ADAPTOR PROTEIN TOM1. Mary K. Brannon<sup>1</sup>, Shuyan Xiao<sup>1</sup>, Geoffrey Armstrong<sup>3</sup>, Kristen Fread<sup>1</sup>, Carla V. Finkielstein<sup>2</sup>, & Daniel G. S. Capelluto<sup>1</sup>, Protein Signaling Domains and <sup>2</sup>Integrated Cellular Responses Laboratories, Department of Biological Sciences, Virginia Tech, Blacksburg VA, 24061 & <sup>3</sup>Department of Chemistry and Biochemistry, Boulder CO, 80309. Adaptor proteins

facilitate cellular cargo sorting through their ubiquitin-binding domains, such as Tollip and Tom1. The interaction of these two proteins is proposed to be involved in the lysosomal degradation of polyubiquitinated cargo. In this work, we demonstrate that binding of Tollip to PtdIns(3)P is negatively modulated by interaction with Tom1. Structural studies determined that the Tom1-binding domain (TBD) of Tollip is intrinsically disordered and folds upon binding to the Tom1 GAT domain, which also undergoes a conformational change upon binding. Intermolecular NOEs of the Tollip TBD-Tom1 GAT complex indicate that association is mainly driven by hydrophobic contacts with very high affinity. Ubiquitin binds to the Tom1 GAT domain at a site that does not overlap with that for the Tollip TBD, but the binding events are mutually exclusive and are driven by conformational changes in the GAT domain, Endosomal localization of Tom1 depends on the presence of Tollip in this compartment. Using fluorescence microscopy, we show that mutations in the binding interphase of the Toml GAT and Tollip TBD complex leads to a dissociation of the proteins and triggers cytosolic localization of Tom1. Consequently, we propose that association of Tom1 to Tollip helps to release Tollip from endosomal membranes, allowing Tollip to commit to endosomal ubiquitinated cargo trafficking.

ELECTRON MICROSCOPY STUDIES OF REP68-AAVS1 COMPLEX. Francisco Zarate-Perez, Montserrat Samso, & Carlos R. Escalante, Department of Physiology and Biophysics, Virginia Commonwealth University, 1101 East Marshall Street, Richmond, VA 23298-0551. Rep68 is a multifunctional protein of the Adeno-associated virus (AAV), a parvovirus widely used as a gene therapy vector, Recognition of the Repbinding site (RBS) by the N-terminal origin-binding domain (OBD) leads to the assembly of a Rep78/Rep68-DNA complex in a process that is highly cooperative. The stoichiometry and nature of this complex is not known. In this work we used a combination of Electron microscopy (EM), single reconstruction, and image analyses to determine the three-dimensional structure of Rep68-AAVS1 complex. We obtained a model that suggests a 7-ring shape structure, which exhibit defined monomer units conforming the molecule. The rings exhibit dimensions of 180 Å X 140-150 Å, and a 34-Å diameter central cavity that appears to be constant at both sides of the particle and is enough wide for the DNA insertion of the DNA. We perform a combination of modeling and fitting using both OBD and Helicase crystallographic structures into the EM model to analyze the EM structure at an atomic level, thus allowing a localization of each domain in the individual monomers. The structure illustrates the remarkable flexibility of Rep proteins to form a diverse number of oligomeric complexes.

# **AWARDS**

# 92<sup>nd</sup> Annual Meeting Best Paper Awards

# AGRICULTURE, FORESTRY, and AQUACULTURE

Best Student Oral Presentation:

**Brittany B. Rice**, Dept. of Animal & Poultry Sciences, Virginia Tech. Insulin-induced hypoglycemia is associated with gene expression changes in the liver and hypothalamus of chickens selected for low or high body weight.

Honorable Mention - Student Oral Presentations:

**Betty R. McConn**, Neuroendocrinology Laboratory, Dept. of Animal & Poultry Sciences, Virginia Tech. Elucidating gonadotropin-inhibitory hormone's mechanism of hunger stimulation.

Louis Nottingham, Dept. of Entomology, Virginia Tech. Investigating the movement and host selection of Mexican bean beetle, Epilachna varivestis Mulsant, among five host plants using mark-release-recapture.

Matthew Rhodes, Dept. of Biology, Eastern Mennonite University. Blueberry antioxidant analysis: Significance of soil treatment and cultivar selection on antioxidant concentrations in highbush blueberries.

### ASTRONOMY, MATH and PHYSICS with MATERIAL SCIENCE

Best Student Oral Presentation and CARPENTER AWARD:

**Quinton Rice**, Advanced Center for Laser Science & Spectroscopy, Dept. of Physics, Hampton University. Temperature- and time-resolved spectroscopy of CuInS2 semiconductor nanocrystals with ZnS capping.

### BIOLOGY with MICROBIOLOGY and MOLECULAR BIOLOGY

Best Graduate Student Oral Presentation and REMSBURG AWARD:

Carly A. York, Dept. of Biology, Old Dominion University. Lateral line analogue aids vision in predator evasion for brief squid (Lolliguncula brevis).

Best Undergraduate Student Oral Presentation:

**Ryan N. Montalvo**, Dept. of Biology & Chemistry, Liberty University. QseC expression regulates flhDC motility of Escherichia coli to increase colonization within the intestine.

Best Student Poster Presentation:

Amanda Hazy, Dept. of Biology & Chemistry, Liberty University. Comparison of brain and blood expression patterns in an Alzheimer's disease mouse model.

Honorable Mention - Student Poster Presentations:

**Ekaterina Marakasova**, School of Systems Biology, George Mason University. PrePs analysis of viral proteins potentially prenylated by human cells.

**Hannah J. Drown**, Dept. of Biology & Chemistry, Liberty University. Antibiotic dynamics of Escherichia coli populations in a streptomycin-treated mouse model.

### BIOMEDICAL and GENERAL ENGINEERING

Best Student Oral Presentation:

**Shirae K. Leslie**, Dept. of Chemical and Life Science Engineering, Virginia Commonwealth University. Controlled release of rat-adipose derived stem cells from alginate microbeads.

### BOTANY

Best Student Oral Presentation and BAKER AWARD:

**Kristina Kowalski**, Dept. of Organismal & Environmental Biology, Christopher Newport University. Vegetation patterns and hydrologic regimes in the Great Dismal Swamp.

Best Student Oral Presentation and HARVILL AWARD:

**Shushil K. Paudyal**, Dept. of Biological Sciences, Old Dominion University. An updated phylogeny of the tribe Chiococceae (Rubiaceae): New insights into taxonomic relationships.

### CHEMISTRY

Best Student Oral Presentation and BRUNER AWARD:

Carla S. McKinney, Center for Materials Research, Norfolk State University. The effect of the presence of Ag on HOMO LUMO gaps in oligomers of thiophene.

### MEDICAL SCIENCES

Best Student Oral Presentation:

Megan A. O'Brien, Dept. of Pharmacology & Toxicology, Virginia Commonwealth University. Alterations to the synaptic transcriptome in response to ethanol-induced sensitization.

Honorable Mention - Student Oral Presentation:

Malaika Argade, Dept. of Medicinal Chemistry, Virginia Commonwealth University. Quinazolines as novel human OCT3 inhibitors: Structure-activity studies.

Best Student Poster Presentation:

**Aravind R. Gade**, Dept. of Pharmacology & Toxicology, Virginia Commonwealth University. Chronic morphine enhances nicotine responses in single mouse enteric neurons.

Honorable Mention - Student Poster Presentations:

**Mohammed Al Mohaini**, Dept. of Pharmacology & Toxicology, Virginia Commonwealth University. Development of natural cyclic peptide inhibitors of XRCC4/XLF interaction for radio-sensitization of breast tumor cells.

**Kevin Tavakolian**, Center for the Study of Chronic Metabolic Diseases, George Mason University. Adiponectin expression in patients suffering from metabolic disorders.

### NATURAL HISTORY and BIODIVERSITY

Best Graduate Student Oral Presentation and WOOLCOTT AWARD:

**Erin Heller**, Dept. of Biology, Old Dominion University. The effects of urbanization on the relationship among birds, ticks, and tick-borne pathogens.

Best Undergraduate Student Oral Presentation and WOOLCOTT AWARD:

**Bryan Finch**, Dept. of Biological Sciences, University of Mary Washington. Using trail cameras to monitor wildlife in Stafford County, Virginia.

### PSYCHOLOGY

Best Student Poster Presentation and RAY KIRBY AWARD:

Patrick Weaver, Center for Health Disparities, Virginia Commonwealth University. Examination of the relation among family resources, adherence and glycemic control in adolescents with Type 1 diabetes.

### STATISTICS

Best Student Oral Presentation:

**Pooja Sengupta**, Dept. of Mathematics & Statistics, Old Domion University. Bivariate doubly-inflated Poisson and related regression.

Honorable Mention - Student Oral Presentation:

Olushina Olawale Awe, Dept. of Statistics, Virginia Tech. A time-varying parameter state space model for the Nigerian economy.

### STRUCTURAL BIOLOGY, BIOCHEMISTRY and BIOPHYSICS

Best Student Oral Presentation:

**Xiaolin Zhao**, Protein Signaling Domains, Dept. of Biological Sciences, Virginia Tech. Structural basis of membrane targeting of the innate immunity adaptor TIRAP.

Best Student Poster Presentation:

Kenneth Lawrence, Dept. of Microbiology & Immunology, Virginia Commonwealth University. Using colocalization assays to define suppressor of IKK-epsilon function.

# JUNIOR ACADEMY AWARDS

# 73rd Virginia Junior Academy of Science Meeting Awards

# ANIMAL AND HUMAN SCIENCES A

Honorable Mention	Matthew Jaffe	Thomas Jefferson Middle
		School
Honorable Mention	Andrew Jordan	George H. Moody Middle
		School
Honorable Mention	Rujul Kaul	George H. Moody Middle
		School
Third Place	Michael Lenzi	Oak Knoll Middle School
Second Place	Alexis Long	George H. Moody Middle
	· ·	School
First Place	Ankit Kumar	George H. Moody Middle
		School

# ANIMAL AND HUMAN SCIENCES B

Honorable Mention	Sarah Purrington	Sabot at Stony Point
Honorable Mention	Leila Shepard	Tuckahoe Middle School
Third Place	Shaandro Sarkar	George H. Moody Middle
		School
Second Place	Lauren Murtha	George H. Moody Middle
		School
First Place	Farheen Zaman	George H. Moody Middle
		School

# **BOTANY**

Honorable Mention	Caroline Lancaster	Chesapeake Bay Governor's
		School
Honorable Mention	Hannah Rennolds	Chesapeake Bay Governor's
		School
Third Place	Anna Polise	Washington-Lee High School
Second Place	John White	Southwest Virginia
		Governor's School
First Place	Gloria Wyszynski	Chesapeake Bay Governor's
	ý ý	School

Second Place

Second Place

CHEMI	CAL	SCH	ENCES	A
V THEY IVE				-

Honorable Mention Aashri Aggarwal George H. Moody Middle

School

Honorable Mention Ava Cordero George H. Moody Middle

School

Third Place Katherine Brickley Tuckahoe Middle School Second Place

Emily Dai George H. Moody Middle

School

First Place Zoe Costello George H. Moody Middle

School

# CHEMICAL SCIENCES B

Honorable Mention Jarrett Gouldin Tuckahoe Middle School Honorable Mention Joshua Haueis George H. Moody Middle

School.

Honorable Mention Calder Lowenthal Swanson Middle School Third Place Annagrace Hamson Tuckahoe Middle School

> Kelsey Levine George H. Moody Middle

School

First Place Wyatt Mingus Sabot at Stony Point

### CHEMICAL SCIENCES C

Honorable Mention Franklin Pugh Tuckahoe Middle School

Third Place Audra White Scott Memorial Middle

School

Catherine Nelli

First Place Nathaniel Sperry Mary Ellen Henderson Middle

School

# CHEMISTRY A

Honorable Mention Cassia Block Washington-Lee High School Honorable Mention Matthew Dabu Washington-Lee High School

Honorable Mention Amirah Anderson Mills E. Godwin Specialty Center for Science, Math &

Technology

Tuckahoe Middle School

Third Place Caitlin Cain Gloucester High School

Second Place Emnet Atlabachew Washington-Lee High School

First Place Christopher Bolton Atlee High School

# JUNIOR ACADEMY AWARDS

# CHEMISTRY B

Honorable Mention	Milen Mehari	Washington-Lee High School
Honorable Mention	Sasha Volodin	Washington-Lee High School
Honorable Mention	Gail Muggill & Natalie Skoloda	Washington-Lee High School
Third Place	Kaitlin Saunders	Chesapeake Bay Governor's School
Second Place	Praveen Ravishankar	Mills E. Godwin Specialty Center for Science, Math & Technology
First Place	Casey Spellman	Washington-Lee High School

# **ECOLOGY & EARTH SCIENCES A**

Honorable Mention	Rachel Alexander	George H. Moody Middle
		School
Honorable Mention	Sam Colaccino	Kenmore Middle School
Honorable Mention	Dylan Klapper	Thomas Jefferson Middle
		School
Third Place	Minoti Kishor	George H. Moody Middle
		School
Second Place	Aditya Kannoth	George H. Moody Middle
		School
First Place	Anupama Jayaraman	Robert Frost Middle School

# **ECOLOGY & EARTH SCIENCES B**

Honorable Mention	Aadi Sharma	George H. Moody Middle
Honorable Mention	Jonathan Spears	School George H. Moody Middle
		School
Honorable Mention	Lilly Yowell	Tuckahoe Middle School
Third Place	Clayton Reppert	Gunston Middle School
Second Place	Ian Street	Tuckahoe Middle School
First Place	Hannah Parker	Sabot at Stony Point

# **ENGINEERING**

Honorable Mention	Michael Bambha	West Potomac High School
Honorable Mention	Lauren Berry	Yorktown High School
Honorable Mention	Seth Peacock	Lee Davis High School
Third Place	Michelle White	Mountain Vista Governor's
		School

#### 108 VIRGINIA JOURNAL OF SCIENCE

Second Place Nicholas Anselmo New Horizon's Governor's

School

First Place Mountain Vista Governor's Andy Page

School

### ENGINEERING & MATHEMATICS A

Honorable Mention Leo Desruelle Williamsburg Middle School Honorable Mention George H. Moody Middle Amy Kaplan

School

Honorable Mention Andrew Logan Tuckahoe Middle School Third Place

Akankshya Jena George H. Moody Middle

Second Place Isabella Dula George H. Moody Middle

School

First Place Naren Kasinath George H. Moody Middle

School

# ENGINEERING & MATHEMATICS B

Honorable Mention William Parker George H. Moody Middle

School

Honorable Mention Taha Shaikh George H. Moody Middle

Honorable Mention Sudarshan Sridharan George H. Moody Middle

School

Third Place George H. Moody Middle Yug Rao

School

Second Place Philip Maruri Tuckahoe Middle School

First Place Nikhil Paul George H. Moody Middle

School

# ENVIRONMENTAL SCIENCE A

Honorable Mention Zephren Collinson Washington-Lee High School

& Justin Szczerbinski

Honorable Mention Sarah Eacho Chesapeake Bay Governor's

School

Honorable Mention Laura Ellis Chesapeake Bay Governor's

School

Third Place Katherine French Chesapeake Bay Governor's

School

Second Place Ronald Cutler Chesapeake Bay Governor's

> & John DeGaetani School

First Place

Colin Fenster & Jack Lohmann Douglas S. Freeman High

School

School

#### ENVIRONMENTAL SCIENCE B

Honorable Mention James Kelley Chesapeake Bay Governor's School Honorable Mention Nargilmaa Khangarid Washington-Lee High School Honorable Mention Adrian Matthews Washington-Lee High School Third Place Washington-Lee High School Johanna Klein Second Place Callie Morgan Chesapeake Bay Governor's School First Place Chesapeake Bay Governor's Nicholas Hipple

#### ENVIRONMENTAL SCIENCE C

Honorable Mention	Thomas Sheehy & Helen Weierbach	Washington-Lee High School
Honorable Mention	Jennifer Radcliffe	Chesapeake Bay Governor's School
Honorable Mention	John Ryan	Hanover High School
Third Place	Elizabeth Ransone	Chesapeake Bay Governor's School
Second Place	Alexandra Pitman	Chesapeake Bay Governor's School
First Place	Catherine Pollack	Mountain Vista Governor's School

#### **HUMAN BEHAVIOR A**

Honorable Mention	Morgan Canaan	Tuckahoe Middle School
Third Place	Vanessa Bethke	Sabot at Stony Point
Second Place	Cordelia Collinson	Kenmore Middle School
First Place	Luke Hawkins	Sabot at Stony Point

#### HUMAN BEHAVIOR B

Honorable Mention	Scott Moss	Tuckahoe Middle School
Honorable Mention	Reona Pereira	George H. Moody Middle
		Sahaal

Honorable Mention	Megan Sundberg	George H. Moody Middle
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School

Third Place Emma Mewborn George H. Moody Middle

School

Second Place Yashodhara Varma George H. Moody Middle

School

First Place Rachel Zhao George H. Moody Middle

School

### MATHEMATICS, STATISTICS & COMPUTER SCIENCE

Honorable Mention	Alexis Brown	Southwest Virginia
		Governor's School

Honorable Mention Spencer Philps Washington-Lee High School

Honorable Mention David Qin Mills E. Godwin Specialty
Center for Science, Math &

Technology

Third Place Michelle Howard Washington-Lee High School

Second Place Mihir Paithane Mills E. Godwin Specialty
Center for Science, Math &

Technology

First Place Jonathan Towne Thomas Jefferson High

School for Science &

Technology

### MEDICINE & HEALTH

Honorable Mention	Arisa Kano	Mills E. Godwin Specialty
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Center for Science, Math &

Technology

Honorable Mention Jennifer Lin Douglas S. Freeman High

School

Honorable Mention Sneha Reddy Mills E. Godwin Specialty

Center for Science, Math &

Technology

Third Place Jake Cui & John Han Thomas Jefferson High

School for Science &

Technology

Second Place Juliana Bain Thomas Jefferson High

& Deboshree Das School for Science &

Technology

First Place Sydney Fain Southwest Virginia

Governor's School

# MICROBIOLOGY & CELL BIOLOGY

Honorable Mention	Mark Feinberg	Washington-Lee High School
Honorable Mention	Elissa Fink	Southwest Virginia
		Governor's School
Honorable Mention	Natalie Heckman	Washington-Lee High School
Third Place	Kevin Zhan	Mills E. Godwin Specialty
		Center for Science, Math &
		Technology
Second Place	Pranay Nuvvala	Mills E. Godwin Specialty
		Center for Science, Math &
		Technology
First Place	Eric Teichner	Mills E. Godwin Specialty
		Center for Science, Math &
		Technology

### PHYSICAL SCIENCE A

Honorable Mention	Lauren Bruns	George H. Moody Middle School
Honorable Mention	Christopher Dalla Valle	George H. Moody Middle School
Third Place	Alexander Daglish	Sabot at Stony Point
Second Place	Bryce Edwards	Gildersleeve Middle School
First Place	Liam David	Swanson Middle School

#### PHYSICAL SCIENCE B

Honorable Mention	Maura Graff	George H. Moody Middle School
Honorable Mention	Lee Martin	Sabot at Stony Point
Honorable Mention	Andrew Mottley	Tuckahoe Middle School
Third Place	Michael Moran	George H. Moody Middle School
Second Place	Max Judish & Justin Lasker	Williamsburg Middle School
First Place	Hunter McGuire	Sabot at Stony Point

### PHYSICAL SCIENCE C

Honorable Mention	Catherine Qian	George H. Moody Middle
		School
Honorable Mention	Ethan Schlussel	George H. Moody Middle
		School

Honorable Mention	Kelton Williams	Thomas Jefferson Middle
		School
Third Place	John Vithoulkas	Tuckahoe Middle School
Second Place	Jacob Yirga	George H. Moody Middle
		School
First Place	Radu Teodorescu	Williamsburg Middle School

### **PHYSICS**

Honorable Mention	Bridget Hart	Yorktown High School
Honorable Mention	Shivam Mehta	Mountain Vista Governor's School
Third Place	Shannon Khurana	Mountain Vista Governor's School
Second Place	Goutham Thiagarajan	Mills E. Godwin Specialty Center for Science, Math & Technology
First Place	Swapnil Pande	Mills E. Godwin Specialty Center for Science, Math & Technology

# PLANT SCIENCES AND MICROBIOLOGY A

Honorable Mention	Skylar Brodowski	Gunston Middle School
Honorable Mention	Jaideep Damle	George H. Moody Middle School
Honorable Mention	Mohini Johri	George H. Moody Middle School
Third Place	Raghda Labban	George H. Moody Middle School
Second Place	Nikhil Chandravel	Tuckahoe Middle School
First Place	Alison Bekenstein	George H. Moody Middle School

### PLANT SCIENCES AND MICROBIOLOGY B

Honorable Mention	Melina Seng	Williamsburg Middle School
Honorable Mention	Reilly Towns	Tuckahoe Middle School
Honorable Mention	Jonathan Yu	Tuckahoe Middle School
Third Place	Selik Morishetty	George H. Moody Middle
		School
Second Place	Joseph Mistretta	George H. Moody Middle
		School

First Place Jerry Zhang George H. Moody Middle School

PSYCHOLOGY		
Honorable Mention	Samuel Havron	Mountain Vista Governor's School
Honorable Mention	Virginia Jarvis	Chesapeake Bay Governor's School
Honorable Mention	Claire Veasey	Southwest Virginia Governor's School
Third Place	Emily Brown	Chesapeake Bay Governor's School
Second Place	Zoe Pettler	Mountain Vista Governor's School
First Place	Abigail England	Washington-Lee High School

# **ZOOLOGY**

Honorable Mention	Grace Grossen	Chesapeake Bay Governor's School
Honorable Mention	Andrew Miller	Mills E. Godwin Specialty Center for Science, Math &
		Technology
Honorable Mention	Kaya Peterson	Mills E. Godwin Specialty
		Center for Science, Math &
		Technology
Third Place	Peter Huson	Washington-Lee High School
Second Place	Kendra Hale	Southwest Virginia
		Governor's School
First Place	Emma Mayes	Mathematics & Science High
		School at Clover Hill

# **Special Awards and Scholarships**

#### VAS BOTANY AWARD

The award is given by the Botany Section of the Virginia Academy of Science, to the best paper on a botanical subject.

> Gloria Wyszynski Chesapeake Bay Governor's School

#### RODNEY C. BERRY CHEMISTRY AWARD

The Rodney C. Berry Chemistry Award is given for the paper that evidences the most significant contribution in the field of chemistry.

> Christopher Bolton Atlee High School

#### WILLIAM W. BERRY ENGINEERING AWARD

This award is given by Dominion Virginia Power in honor of Mr. William W. Berry who was a past Chairman of the Board of Virginia Power.

> Andy Page Mountain Vista Governor's School

#### ANN M. HANCOCK CELLULAR BIOLOGY AWARD

This award is given to the best paper in cellular biology and is presented in memory of Anne M. Hancock who retired from Patrick Henry High School in Hanover County and who gave many years of service to the Jr. Academy not only by teaching but also serving on the Jr. Academy Committee.

Eric Teichner

Mills E. Godwin Specialty Center for Math, Science & Technology

#### ROSCOE HUGHES GENETICS AWARD

The Roscoe Hughes Award is given for the best paper in the field of Genetics. Emma Mayes

Mathematics & Science High School at Clover Hill

#### MATHEMATICS AWARD

The Mathematics Award is given for the paper that evidences the most significant contribution in the field of Mathematics.

> Michelle Howard Washington-Lee High School

#### STATISTICS AWARD

The Statistics Award is given for the paper that evidences the most significant contribution in the field of Statistics.

David Qin

Mills E. Godwin Specialty Center for Science, Math & Technology

#### VABE ZOOLOGY AWARD

This award is presented by the Virginia Association of Biology Educators and is given for outstanding research in the Zoology section.

Emma Mayes

Mathematics & Science High School at Clover Hill

#### CANCER RESEARCH AWARDS

These awards are to recognize outstanding science papers related to cancer research. These awards are provided by the American Cancer Society.

Third Place Jerry Zhang Second Place Jake Cui First Place Andrea Gayle

#### DR. AND MRS. PRESTON H. LEAKE AWARD IN APPLIED CHEMISTRY

The Dr. and Mrs. Preston H. Leake Award in Applied Chemistry is given to the author of a research paper which best exemplifies how chemicals, chemical principles, or chemistry have been used, are used, or might be used to enhance or even to save life.

Kaitlin Saunders Essex High School

#### INFECTIOUS DISEASES AWARD

This award is given to commemorate Dr. Smith Shadomy by the Virginia Chapter of the National Foundation for Infectious Diseases to the paper that evidences outstanding research in the field of infectious diseases.

Andrea Gayle

#### SPELEOLOGICAL SOCIETY AWARD

Given by the Richmond Area Speleological Society for outstanding research addressing karst or topics related to speleology (bats, caves, carbonate geology, paleoenvironments, limestone fossils, sinkholes, etc.

(No awards this year)

#### VIRGINIA MUSEUM OF NATURAL HISTORY (VMNH) AWARD

This award is presented by the Friends of the Virginia Museum of Natural History in recognition of a significant contribution in the study and interpretation of Virginia's Natural Heritage.

Elizabeth Ransone

#### VIRGINIA SEA GRANT COLLEGE PROGRAM AWARD

This award is given by the Virginia Sea Grant College Program for outstanding marine or coastal research.

Katherine Steward French Chesapeake Bay Governor's School – Warsaw Campus

#### GAMMA SIGMA DELTA AWARD

The Gamma Sigma Delta Award is presented by the Virginia Tech Chapter of the Honor Society of Agriculture. This award is given in recognition of excellence in research dealing with application of new technologies and/or concepts in agriculture forestry, or veterinary medicine.

Laura Ellis Chesapeake Bay Governor's School

#### BETHEL HIGH SCHOOL SCHOLARSHIP

This \$1,000 Scholarship Award comes from the interest earned from a \$10,000 endowment contributed by the students of Bethel High School, Hampton, Va., over a two year period. This award is based on both the student's presentation and paper.

Catherine Pollack

Mountain Vista Governor's School for Science, Mathematics & Technology

### HENRY MACKENZIE ENVIRONMENTAL SCHOLARSHIP

This \$5,000 scholarship will be awarded to the student whose paper evidences the most significant contribution in the field of Environmental Science dealing with the James River Basin and Chesapeake Bay. The Virginia Environmental Endowment and VJAS offer this scholarship in tribute to the outstanding and generous services of Judge Henry W. MacKenzie, Jr., one of the founding directors, who has a great interest in the James River and the Chesapeake Bay.

Jennifer Radcliffe

Chesapeake Bay Governor's School for Marine and Environmental Science

#### FRANCES AND SYDNEY LEWIS ENVIRONMENTAL SCHOLARSHIP

A \$14,000 scholarship (\$3,500 per year for four years) for the best effort by a student in grades 9 to 12 in the field of environmental science. This scholarship is in the name of Frances and Sydney Lewis and is given by the Virginia Environmental Endowment.

Catherine Pollack

Mountain Vista Governor's School for Science, Mathematics and Technology

#### VJAS Honors Awards

#### DOROTHY KNOWLTON AWARD

This award is given to the Middle School student presenting the best paper in the life sciences. It is presented in honor of Dorothy Knowlton, the former Science Coordinator of Arlington County, who was instrumental in advancing experimental design within the science curriculum.

Jerry Zhang George H. Moody Middle School

#### JOYCE K. PETERSON AWARD

This award is given to the Middle School student presenting the best paper in the physical sciences. It is presented in honor of Mrs. Joyce K. Peterson who has been an outstanding teacher in the Arlington County Schools.

Bryce Edwards Gildersleeve Middle School

#### CATESBY JONES AWARD

This award is given to the senior student whose paper evidences the most significant contribution to science.

Sydney Fain
Southwest Virginia Governor's School

#### VAS BEST RESEARCH AWARDS

These awards are given by the Virginia Academy of Science to two papers and two alternate papers, in grades 8-11, presenting the most outstanding papers at the Research Symposium. The winners will be invited to attend the annual meeting and present their papers as a poster presentation before the American Junior Academy of Science (AJAS). The 2015 AJAS meeting will be held in February in San Jose, California.

2nd Alternate Emma Mayes

Mathematics & Science High School at Clover Hill

1st Alternate Abigail England

Washington-Lee High School

Winner Colin Fenster & Jack Lohmann

Douglas S. Freeman High School

Winner Eric Teichner

Mills E. Godwin Specialty Center for Science, Math & Technology

#### ERTLE THOMPSON MEMORIAL ENDOWMENT AWARD

The Ertle Thompson Memorial Endowment Award is established to honor Dr. Ertle Thompson. These awards will be presented annually to the winners of this Symposium, to send them on to the annual meeting of the American Association for the Advancement of Science and the American Junior Academy of Science.

Colin Fenster & Jack Lohmann
Douglas S. Freeman High School
Eric Teichner

F. Godwin Specialty Conton for Science, Math & T.

Mills E. Godwin Specialty Center for Science, Math & Technology

#### DR. R. DEAN DECKER HONORARIUM AWARD

The Dr. R. Dean Decker Honorarium award this year of \$500 each is to go to the teacher/sponsor of the two top student winners of the AJAS trip. The monies can be used at the discretion of the recipients as long as it is related to the attendance at the AJAS meeting. Guidelines and procedures for the recipients will follow later.

WinnerMarissa Vest WinnerDana Delano

#### VAS AWARD FOR DEDICATED SERVICE TO VJAS

The support of VAS makes all of this possible, In addition to several awards that have already been given by VAS, I would like to ask President of VAS, to present the highest service award of the Junior Academy. This award recognizes past VJAS officer or VJAS member who has continued to provide support as volunteer to the Junior Academy even after completion of high school.

Se Jeong

#### VAS HONORARY MEMBERSHIP

This award which includes one year's membership in the Virginia Academy of Science including a subscription to the *Virginia Journal of Science* is awarded for outstanding service to the Junior Academy and the VJAS Committee.

Eileen Breslin

#### **VJAS OFFICERS 2014-2015:**

Officer Alum	Margaret Doyle
Editor-in-Chief & Communications Liaison	Kevin Zhan
Secretary	Jon Gustafson
Vice President	Wyatt Booth
Co-Presidents	Ben Rhoades and Christopher Bolton

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